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ERRATA

Page 108, footnote, "no. 1, p. 1–25, 1 fig." should read "no. 1, p. 1–25, 1912."

Page 111, line 7 from bottom, "Protein bodies stain," etc., should read "These so-called protein bodies stain," etc.

Page 172, Table I, last column, line 13, "9" should read "0."

Page 230, line 12, "methylene violet and with cosin" should read "methylene violet with eosin."

Page 234, line 6, "indicating presence of a reagent" should read "indicating the presence of the reagent."

Page 279 "*Rheosporangium aphanidermatus*" should read "*Rheosporangium aphanidermatum*.

Page 291, line 7, "*Rheosporangium aphanidermatus* n. sp." should read "*Rheosporangium aphanidermatum*, n. sp."

Page 306, line 2 from bottom, "which were treated as noted before" should read ".which were treated as hereafter noted."

Page 344, line 10, "as indicated under 1" should read "as indicated under a."

Page 368, line 2 from bottom, "A new method of enumerating bacteria" should read "A new method of enumerating bacteria in air."

Page 455, legend under figure 3, "center" should read "outer."

Page 477, line 18 from top, "It is Gram-positive" should read "It is Gram-negative."

Page 477, line 23 from top, "*Bacillus lactuacae*" should read "*Bacillus lactucae*."

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PHOMA DESTRUCTIVA, THE CAUSE OF A FRUIT ROT OF THE TOMATO

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OCCURRENCE AND GENERAL APPEARANCE OF THE DISEASE

In March, 1912, specimens of tomatoes (*Lycopersicon esculentum*) affected with a fruit rot were sent in for examination from Cutler, Fla. Mr. James B. Brown, who selected and sent in the specimens, stated in an accompanying letter that great loss had been caused among the farmers of Dade County, Fla., by this fruit-spotting. When received, some of the fruit was green, some ripe, and some just beginning to color. Most of the tomatoes had conspicuous dark spots on the side and at the stem end. These spots, occurring on both green and ripe fruit and measuring 1 to 3 cm. in diameter, were brownish black in color, with definite outlines, while on the surface of the largest spots tiny dark pustules could be seen. The firm, discolored tissue of the spots was somewhat sunken, forming slight depressions, the surface of which was membranous or crustlike, according to the stage of development. On the ripe fruit the dark tissue was surrounded by a more or less watery-looking zone. Plate A, figure 1, shows the fruit-spotting as it appeared upon one of the Cutler specimens.

A microscopic examination of tissue cut from diseased areas occurring on both green and ripe tomatoes showed a dense network of fungous mycelium within the cells. The hyphæ were septate, branched, and hyaline to brown in color, while scattered over the surface of the mycelial growth numerous small dark pycnidia could be seen (Pl. I, fig. 1). These pycnidia, varying considerably in size, were for the most part round in outline, with a distinct central pore, out of which issued masses of hyaline 1-celled spores in long coils. The structure of a pycnidium with its relation to the host cells is shown in Plate I, figure 1, and a few single hyphal strands in Plate I, figure 2. Bacteria were also numerous in the tissue of the diseased area, and in some of the spots spores of

Macrosporium were observed. Since, however, the pycnidial fungus, whose general appearance indicated that it belonged to the genus Phoma, was found in such abundance in tissue from all of the spots examined, it seemed probable that this fungus might prove to be the primary cause of this tomato fruit-rot. An isolation of this fungus from diseased tissue was therefore undertaken.

ISOLATION OF THE FUNGUS FROM DISEASED TOMATO FRUIT

With sterilized instruments small pieces of diseased tissue, partially sterilized in a solution of mercuric chlorid (1 to 1,000), were cut out from beneath the surface of several of the spots and transferred to cultural media. A grayish white mycelial growth developed on string-bean agar, and at the end of two weeks small dark pycnidia similar to those found on the tomato fruit were observed. Under the microscope numerous hyaline 1-celled vacuolated spores could be seen issuing from the crushed pycnidia. Some of these spores were transferred to sterilized potato cylinders, cotton and tomato stems, lima-bean, prune, and string-bean agars, and from these transfers pure cultures of the Phoma fungus were obtained and later used in inoculation experiments. Pycnidia and spores were produced in abundance on potato cylinders, tomato stems, and upon lima-bean and prune-agar slants.

Nearly two years after the receipt of the Cutler specimens some diseased tomatoes affected with a similar spotting were sent in (February, 1914) by A. F. Young & Co., New York City. These tomatoes were selected from shipments received from Cuba (Pl. III, fig. 1) and from Punta Gorda, Fla. By the usual agar plate methods a Phoma fungus, identical with that isolated from the Cutler fruit, was obtained from tissue cut from spots occurring upon specimens from both Cuba and Florida.

INOCULATION EXPERIMENTS

INFECTION OF GREEN AND RIPE FRUIT IN THE GREENHOUSE

Inoculation experiments were begun on March 30, 1912, with some large tomato plants growing in the greenhouse. Needle-prick inoculations were made into green and ripening fruit, and also into the stems and leaves of these plants, the Phoma fungus being transferred from 20-day-old cultures of string-bean agar. Plants having fruit, stem, and leaves pricked with a sterilized needle were used as controls. In four to six days spots developed on green and ripe tomatoes, occurring upon the sides, stem end, and blossom end of the fruit, according to the position of the inoculation pricks. The spots thus produced by inoculation of the fungus were similar in appearance to those observed upon the diseased specimens from Florida. A week after inoculation the stem tissue of the tomato plant was seen to be slightly discolored about the needle pricks. This discoloration, however, had not spread to any considerable extent, and the surrounding tissue still remained

firm. Cross sections of a stem cut through the point of inoculation showed a brownish area, with mycelium in the epidermal and outer vascular tissue. On the leaves no evidence of spotting was observed two to four weeks after inoculation.[1] At the end of two weeks the spots produced on the fruit by inoculation had spread considerably beyond the needle pricks, and the tissue had perceptibly darkened. The ripe tomatoes were beginning to fall from the vines. Some of these inoculated specimens were brought to the laboratory for examination. The almost black spots, measuring 2 to 3.5 cm. in diameter on the ripe fruit, were surrounded by a lighter zone the tissue of which was soft and somewhat sunken, while that of the central portion of the spot was black and crustlike. Tissue from the discolored areas was examined and found to be full of fungus mycelium, while on the surface of the crustlike portion numerous dark pycnidia could be seen. The hyaline to brownish hyphæ were septate and branched, and the dark spherical pycnidia contained masses of 1-celled hyaline spores which issued in coils from a central pore. A microscopic study of this fungus, made from diseased tissue taken from inoculated fruit spots, showed it to be the same as that used in the inoculation and previously isolated from the Cutler material.

The spots produced on the green fruit two weeks after inoculation were not as large as those of the ripe tomatoes, measuring only 1 to 1.5 cm. in diameter, and the discoloration presented a somewhat different appearance. The central portion of the spot on the green fruit was of lighter color, becoming darker toward the circumference and merging into a definite dark-brown border.

A second inoculation experiment with greenhouse tomato plants was begun on April 17, 1912, when green and partly ripened fruits were inoculated by means of needle pricks with fungus from 22-day-old tomato-stem cultures. Two weeks after inoculation conspicuous dark diseased areas had developed, similar in appearance to the spots produced by the previous inoculation and to those of the diseased Florida tomatoes. Examination of the darkened tissue showed mycelium, pycnidia, and spores of the fungus inoculated.

An interruption of the work occurred at this time, and further study of this tomato fruit rot was not resumed until the spring of 1913. Cultures of the Phoma fungus were kept growing upon different kinds of media, transfers being made every 8 to 10 weeks. In February, 1913, the work was resumed. Questions as to how early the fungus is able to infect the fruit and the means by which it gains entrance to the tissue suggested themselves, and experiments in greenhouse and laboratory were begun in order to determine something definite in regard to these points.

[1] Later experiments proved the fungus to be parasitic under certain conditions of moisture and temperature upon both leaves and stems of the tomato. Failure to produce the disease in the early experiments is believed to have been due to the low humidity and temperature of the atmosphere of the greenhouse.

Twelve young tomato plants, 30 to 40 cm. high, blossoming and with fruit set, were selected for one experiment. The plants were growing in pots in the greenhouse and were in a healthy condition.

A fungous suspension was made by pouring 10 c. c. of sterilized water into 53-day-old cotton-stem cultures of the fungus which were rich in pycnidia and spores. The culture tubes were well shaken, after which the suspension was poured off into clean tubes.

Three methods of inoculation were used:

(1) The spore suspension was transferred by means of a camel's-hair brush to the fruit of two plants. Care was taken not to injure the tissue of one plant while needle pricks were made into the fruit of the other.

(2) By means of an atomizer the suspension was sprayed upon four plants, thoroughly wetting the fruit. Two of these plants were not wounded and two were injured after spraying by pricking the fruit with a sterilized needle.

(3) Direct needle-prick inoculations from cotton-stem cultures of the fungus were made into the fruit of three plants.

Three plants having fruit pricked with a sterilized needle were set aside as controls. During the first 24 hours these 12 plants were protected from sunlight by strips of thick manila paper. Observations were made every few days and records taken at the end of 6, 12, 24, and 28 days. The results are given in Table I.

TABLE I.—*Results of inoculations of young green tomato fruit with Phoma fungus (greenhouse experiment)*

Method of inoculation.	Results of inoculation			
	After 6 days.	After 12 days.	After 24 days.	After 28 days.
Spore suspension transferred with brush.	No infection of fruit not wounded. Slight discoloration of tissue upon needle-pricked green fruit.	No infection of fruit not wounded. Discoloration spreading about pricks on green fruit.	Tomatoes beginning to turn red. No infection of fruit not wounded. Definite spots forming on needle-pricked fruit.	Tissue of ripening tomatoes firm, with no evidence of infection of fruit not wounded. Diseased spots produced about needle pricks.
Spore suspension sprayed on fruit.	No infection of fruit not wounded. Discoloration of tissue on needle-pricked green fruit.	No infection of fruit not wounded. Discoloration spreading about inoculation pricks at stem end, blossom end, and sides of green fruit.	No infection of fruit not wounded. Definite spots about needle pricks. Pycnidia forming on center of spots.	Tissue firm with no evidence of infection on fruit not wounded. Diseased spots produced about needle pricks.
Inoculation by means of needle pricks.	Brownish black discoloration of tissue about inoculation pricks on green tomato.	Definite brownish black spots developed about all inoculations on green fruit.	Spots 2 to 4 cm. in diameter, with black central portion, brownish circumference, and watery band surrounding. Pycnidia scattered over central portion.	Conspicuous diseased spots produced about needle-prick inoculations.
Check plants. Fruit pricked with sterilized needle.	No infection.....	No infection.....	No infection.....	No infection.

Four weeks after inoculation the tomatoes which had been exposed without injury to the spraying of the spore suspension in the foregoing experiment were picked and placed in moist chambers in the laboratory. This was done in order to see whether infection would occur on the sprayed fruit when separated from the vine. At the end of 12 days the tissue of these tomatoes remained firm and there was no evidence of infection.

Fully grown tomato plants bearing green and ripening fruit were used in another experiment in order to show the effect of fungous inoculations by the wounding and nonwounding of nearly mature tomatoes. Transfers with a platinum loop were made from tomato-stem cultures of the Phoma fungus, to blossom end, stem end, and sides of green and ripening fruit. Some of the tomatoes were needle-pricked and some were not injured. Control plants were also used. The observations given in Table II were made 5 and 10 days after inoculation.

TABLE II.—*Results of the inoculation of maturing tomato fruit with Phoma fungus (greenhouse experiment)*

Method of inoculation.	Results of inoculation.	
	After 5 days.	After 10 days.
Fungus transferred to surface of fruit. No wounding.	No discoloration at stem end, blossom end, or on sides of fruit.	No infection from inoculations.
Fungus transferred to surface of fruit, wounding by needle pricks.	Discoloration about needle pricks at stem end, blossom end, and sides of fruit.	Diseased spots produced about pricks with characteristic discoloration and decay.
Check tomatoes pricked with sterilized needle.	Free from infection.....	Free from infection.

As a result of inoculations into young and maturing tomato fruits, it may be seen from data in Tables I and II that infection begins soon after the fruit sets, a slight discoloration or definite spotting developing within five to seven days, according to the method of inoculation used. It is also apparent that the fungus is a wound parasite, since no infection occurred where the epidermis of the fruit remained unbroken. Wounding of the fruit by insects, bruising, and natural cracking of the tissues no doubt affords a common means of entrance for this parasite.

In the inoculation work it was noticed that the discoloration of the tissue developed rapidly upon ripening tomatoes, the spots measuring 1 to 3 cm. in 10 days, while those upon the green fruit were only about one-fifth as large in the same length of time. The discolored tissue of the ripe fruit varied from brown at the circumference to almost black in the central portion, the whole being bordered by a zone of watery-looking tissue. In the green fruit a lighter central area became surrounded by a darker band, and the watery zone was not noticeable. Numerous pycnidia developed upon the darkest tissue, giving the surface a pimply appearance.

In tomatoes diseased by inoculation with the Phoma fungus the discoloration was found to penetrate from 2 to 3 cm. into the interior of the fruit, and microscopic examination showed an abundance of hyaline to brownish mycelium in the watery-looking tissue, as well as in that of the darker central tissue.

INFECTION OF TOMATO FOLIAGE IN THE GREENHOUSE

As already mentioned in connection with the inoculation of tomato fruit, the Phoma fungus was also inoculated into both stem and leaf tissue of young and mature tomato plants growing in the greenhouse. In a few instances a slight discoloration had been noticed, but no definite spotting had been produced. Up to this time no effort had been made to subject the inoculated plants to a higher temperature or to a more humid atmosphere than that afforded by ordinary greenhouse conditions. In January, 1914, a series of experiments was begun in the greenhouse to determine whether or not leaf infection would occur in plants placed under conditions more favorable to the development of the fungus.

Healthy young tomato plants of the Earliana variety growing in pots in the greenhouse were used. When inoculated, the plants were from 10 to 15 cm. in height. A spore suspension of the Phoma fungus, made by adding sterile water to a corn-meal culture containing an abundance of mature pycnidia, was transferred by means of a platinum loop to the leaf surface. The tissue was then needle-pricked and the plants placed under glass bell jars. Control plants were used. The atmosphere beneath the jars soon became saturated, and drops of water collected on the inside of the jar and upon the surface of the plant. In three to five days a discoloration of the leaf tissue was noticed about the points of inoculation, and in eight days definite dark spots had developed. These spots varied in size from 2 to 10 mm. In some cases several spots had coalesced, spreading across the entire leaflet. Photographs were made of two of these diseased plants (Pl. II, figs. 3 and 4). A microscopical examination of tissue taken from these spots showed the mycelium, pycnidia, and spores of the Phoma. From diseased tissue cut from some of the spots agar plates were poured and the fungus reisolated in pure culture.

A second experiment with young tomato plants was tried, the inoculation being made by spraying with a spore suspension. No needle pricks were made. The plants were placed beneath glass bell jars in the greenhouse, and within five days dark spots had developed (Pl. B, figs. 2 and 3). Small drops of water were noticed on the plants, especially along the edges of the leaves. Frequently the infection started beneath these drops of water, the discoloration spreading from the edge of the leaf inward. Microscopical examination of tissue from the diseased areas showed mycelium and pycnidia of the Phoma fungus.

Control plants placed beneath bell jars and subjected to the same conditions of humidity and temperature as plants used in this and the former experiment gave no evidence of disease. Since young tomato plants had proved susceptible to infection of the fungus under conditions of high temperature and humidity, it was decided to subject mature tomato plants to a similar test. Accordingly two nearly mature tomato plants (90 to 100 cm. high) growing in pots and bearing green fruit were selected and placed in glass infection cages.[1] One of these plants was thoroughly sprayed (January 17, 1914) with a spore suspension of the fungus, made by adding distilled water to a 10-day-old corn-meal culture. Spraying was done with a small atomizer, the foliage, stem, and fruit being thoroughly wet with the solution. The other plant was placed in a similar cage and sprayed with sterile water. In four days small dark spots had appeared on the foliage of the inoculated plant, giving the lower leaves a speckled appearance. A diseased leaf was picked, brought to the laboratory, and examined. It was found that each dark spot represented a point of fungus infection, as many as 50 spots being counted on a single leaflet. By means of beef-agar plates pure cultures of the Phoma fungus were recovered from this diseased leaf tissue. Infection spread rapidly upon the plant in the cage, until in 10 days' time the whole plant was badly spotted. Six of the lowest leaves had fallen from the stem, while those a little higher up were discolored, shriveled, and drooping. The upper leaves which were still green showed dark, irregular spots. Upon some of the petioles dark streaks and blotches occurred. On January 27, 1914 (10 days after inoculation), a photograph was made of the diseased plant taken from the infection cage (Pl. II, fig. 2). The control plant which showed no spotting or falling away of leaves was also photographed (Pl. II, fig. 1). Some of the tissue from the diseased plant was again examined in the laboratory, and plate cultures were made. Out of tissue cut from leaf spots pure cultures were isolated. A few of the leaves which had fallen from the inoculated plant were photographed separately in order to show more clearly the character of the spotting (Pl. IV). Three weeks after inoculation green and partly ripened fruit growing upon this diseased plant showed no signs of disease.

INFECTION OF GREEN AND RIPE FRUIT IN THE LABORATORY

In addition to the greenhouse experiments some infection tests were carried on in the laboratory. Green and ripening tomatoes were picked from healthy plants growing in the greenhouse. This fruit was washed, soaked in mercuric-chlorid solution (1 to 1,000) for 30 minutes, and

[1] The infection cages used had glass tops and sides set in wooden frames. When placed over the inoculated plants, the whole cage was raised about 5 cm. by means of supports, in order to allow ventilation. Capacity of cage, 170,633 c. c.

placed in glass moist chambers. Transfers from a spore suspension of the Phoma fungus were made with a camel's-hair brush to the blossom end, the stem end, and the sides of the fruit, after which some of the tomatoes were needle-pricked, while care was taken not to wound the others. Check tomatoes not treated with the spore suspension but kept in moist chambers under similar conditions were used as controls. Four days after inoculation a darkening of the tissue became visible about the pricks, especially at the stem end of the tomatoes. Where there had been no wounding of the tissue, no discoloration appeared. Ten days after inoculation diseased areas 2 to 3 cm. in diameter had developed from needle-prick inoculations on the sides and at the stem end of the tomatoes. The dark tissue in the central part of the spots was surrounded by a watery-looking zone. Cracking of the discolored tissue had occurred in some cases, and within the cracks a grayish white mycelium could be seen. On tomatoes not wounded at the time of inoculation (and not cracking later) no discoloration appeared. There was, however, in some of the nonpricked tomatoes a cracking of the tissues at the stem end, and in these cases the typical discoloration due to the presence of the fungus was observed.

A final examination made at the end of three weeks showed well-developed diseased spots about needle-prick inoculations, while no spotting occurred upon uninjured tomatoes treated with the fungus, except where natural splitting of the tissue at the stem end allowed the fungus to enter. The check tomatoes showed no evidence of disease. Several of the needle-pricked tomatoes kept in the moist chamber and showing spots at the stem end of the fruit were selected and photographed. (Pl. A, figs. 2, 3, 4, and 5.) Freezing microtome sections were made from tissue taken from fruit spots and the development of pycnidia and pycnospores was studied. Plate I, figure 6, shows the structure of a pycnidium in cross section. Within the darker outer cells are several layers of hyaline cells from which arise the basidia bearing the spores. In Plate I, figure 3, a few of these spores are shown under higher magnification. Table III gives the results of these inoculations.

TABLE III.—*Results of the inoculation of green and ripe tomato fruit with Phoma fungus (laboratory experiment)*

Methods of inoculation.	Results of inoculation.		
	After 4 days.	After 10 days.	After 3 weeks.
Spore suspension transferred with brush to surface of fruit. Needle-pricked.	Darkening of tissue around pricks.	Distinct spots produced about pricks.	Characteristic spotting with pycnidia on the surface.
Spore suspension transferred with brush to surface of fruit. No wounding.	No discoloration of tissue.	No infection.....	No infection, except at stem end, where splitting of tissue occurred.

A laboratory experiment was also tried under thermostatic conditions of temperature and moisture. Green tomatoes picked from plants growing in a garden plot near the pathological greenhouses were thoroughly washed in water, soaked in a 2 per cent formaldehyde solution for three hours, after which needle-prick inoculations were made (Aug. 1, 1914) from a 6-weeks-old culture of the Phoma fungus. The inoculated tomatoes were then wrapped separately in paraffin paper and placed in zinc boxes in different compartments of an incubator. Water in a lower tray kept the air saturated. Temperature records were taken each day and averaged for the period during which the experiment lasted. Uninoculated tomatoes were used as checks. At the end of 18 days the tomatoes kept at an average temperature of 6.6° C. showed no decay and only a slight discoloration of tissue about the needle pricks, while tomatoes kept at 19.7° C. showed decayed spots in every case. Within the tissue of these spots, measuring 2 to 4 cm. in diameter, pycnidia and spores of the Phoma were found in abundance.

INFECTION OF TOMATO PLANTS IN THE FIELD

On June 10, 1914, six tomato plants (Matchless variety) growing in garden plots at Arlington Farm were sprayed with a spore suspension of the Phoma fungus made from a corn-meal culture. On August 15 the plants were examined and a few spotted leaves picked and brought to the laboratory. Pycnidia and spores of a Phoma were found in tissue cut from the spots. A month later the plants were again examined, and the leaves, stem, and fruit were found to be affected. Material was collected and brought in for microscopical study. The brown discolored patches on the leaves had a grayish center, upon the surface of which scattered pycnidia could be seen. From these small brown pycnidia spores issued in coils. Within the discolored tissue of the petiole and stem similar pycnidia and spores were found. The spotting of the fruit was characteristic, and here, too, the Phoma fungus was found.

Experiments were also made on August 27, 1914, with six tomato plants of the Livingston Coreless variety. A spore suspension of the Phoma was used and the fruit was needle-pricked after spraying. On September 10 fruit spots were observed upon green, partly ripened, and fully ripened fruit. By means of agar plates the fungus was reisolated from fruit and stem tissue.

CROSS-INOCULATIONS BETWEEN TOMATO FRUIT AND LEAF

The foliage of young and nearly mature tomato plants was sprayed with a fungous suspension of the Phoma made from cultures isolated from diseased tomato fruit tissue. Diseased spots were produced in four to six days, and the fungus was recovered from the leaf tissue by means of agar plates. With cultures thus obtained needle-prick inoculations were made into green and ripening fruit (greenhouse plants), and the characteristic Phoma spotting was again produced.

OTHER PLANTS EXPOSED TO INFECTION OF THE FUNGUS

A number of experiments were made in order to test the pathogenicity of the Phoma fungus upon eggplant, potato, sugar beet, Jimson weed, garden pea, bean, and pepper plants.

EGGPLANT (*Solanum melongena*).—In February, 1914, young eggplants growing in pots at the greenhouse were sprayed with a spore suspension of the Phoma fungus made from a 4-weeks-old corn-meal culture. Some of the sprayed plants were placed beneath glass bell jars, with cotton-plugged tops, and some were placed in a glass infection cage. Control plants were used in both cases. The bell jars were removed from a few of the plants after 48 hours, but were kept over others for a period of 10 days. At the end of this time numerous small brown spots had appeared on the leaves of the plants kept beneath the bell jars and upon the plants in the infection cage, but no spotting was found upon plants uncovered after 48 hours. Lower leaves of the diseased plants were yellowish and drooping and were the first to show the spotting. Tissue from these spots was examined with the microscope, and pycnidia and spores observed. Spores could be seen issuing from the pycnidia in long coils. The majority of spots remained small in size, only a few becoming 3 to 8 mm. in diameter. The spotted leaves did not fall from the stems, and the inoculated plants continued to grow and to produce new leaves. By means of agar plates the Phoma fungus was recovered from spotted tissue taken from plants kept under bell jars and from sprayed plants in the infection cage. Control plants kept under similar conditions of temperature and moisture showed no spotting. From these experiments it appears that the Phoma isolated from diseased tomato tissue is slightly pathogenic to eggplant,[1] but since spotting occurred only upon plants kept beneath bell jars or within the infection cage for several days, it is not probable that eggplants grown under ordinary conditions of temperature and moisture would become infected with this fungus.

POTATO PLANTS (*Solanum tuberosum*).—Healthy young potato[2] plants 15 to 20 cm. high, growing in pots at the greenhouse, were sprayed on January 28, 1914, with a spore suspension of the Phoma fungus made from a 2-weeks-old corn-meal culture. Some of the plants were covered with bell jars, while others were placed in an infection cage. The bell jars were removed from some of the plants after 48 hours, but were kept over others for a period of 10 days. Within six days discolored spots were noticed upon leaves of plants under the bell jars and upon leaves of those in the infection cages (Pl. B, fig. 1). The lowest leaves showed the

[1] Cultures of the *Phoma* were compared with *Phomopsis vexans* (Sacc. and Syd.) Harter, recently described by L. L. Harter (1914) as causing fruit-rot, leaf-spot, and stem-blight of eggplant. The two fungi were found to be quite distinct morphologically, and according to inoculations made by Harter *Phomopsis vexans* is not parasitic on tomato.

[2] Saccardo reports the following species of Phoma on *Solanum tuberosum*: "*Phoma nebulosa* (Pers.) Mont." (1884, p. 135); "*Phoma eupyrena*" (1884, p. 229); "*Phoma solani* Cooke et Hark." (1892, p. 275); "*Phoma solanicola* Prillieux et Delacroix" (1892, p. 175).

first infection, dark streaks appearing along the petioles and spots upon the leaf surface. Within 12 days infection had become general, spots having been produced upon the youngest leaves as well as upon the oldest. The spots varied from pinpoint in size to areas 5 to 6 mm. in diameter. Frequently these spots united, forming irregular blotches, the tissue within which became dark-colored and shrunken, until finally the whole leaf yellowed and fell from the stem. Diseased leaves picked from plants under bell jars and from plants in the infection cages were brought to the laboratory for microscopical examination. Mycelium, pycnidia, and spores of the Phoma were found, and by means of agar plates the fungus was recovered in pure culture. The inoculated plants which were kept under bell jars for only 48 hours and then exposed to ordinary greenhouse conditions of temperature and moisture showed some spotting on the lower leaves. These spots, however, increased only slightly, and after three weeks the plants appeared to have thrown off the disease and were making good growth. A few of the leaves taken from a plant in the infection cage were photographed on March 20, 1914 (Pl. V). The average maximum temperature of air in the infection cage during 10 days (March 10–20, 1914) was 7.5° C. higher than that of the room, while the average minimum temperature of the cage was 4.1° C. lower than the room temperature.

POTATO TUBERS (*Solanum tuberosum*).—Potato tubers (Irish Cobbler variety) were thoroughly washed, soaked in a solution of mercuric chlorid (1 to 1,000) for two hours, and rinsed in distilled water. Stab inoculations were then made into the tubers from an oat-agar culture of the Phoma fungus, and the potatoes were placed in zinc boxes. The average temperature within the boxes was 25° C. during a period of four weeks, and the humidity was nearly 100 per cent, the boxes being kept wet with moist cotton and filter paper. At the end of eight days a slight darkening of the tissue was noticed about the needle stabs, but at the end of four weeks no definite spots had been produced. Four other inoculation experiments with potato tubers were tried, three in moist chambers at temperatures ranging from 14° to 29° C. with a low humidity and one at a temperature of 35° with high humidity. Under these conditions no infection occurred.

SUGAR-BEET PLANTS (*Beta vulgaris*).—Young sugar-beet plants growing in pots at the greenhouse were inoculated on January 28, 1914. The plants, which were 10 to 16 cm. high, were sprayed with a spore suspension of the Phoma fungus and placed under bell jars. Control plants were used. The plants were examined at intervals of several days, but showed no spotting in a period of three weeks.

Half-grown sugar beets growing in an open bed in the greenhouse were sprayed with a spore suspension of the fungus on February 4, 1914. These plants were not covered. In four weeks no spotting or other evidence of infection had taken place.

JIMSON WEED (*Datura tatula*).—On April 25, 1914, three mature plants of Jimson weed growing in pots at the greenhouse were sprayed with a spore suspension of the Phoma fungus. These plants were placed beneath bell jars for a period of 10 days, but no leaf spotting developed.

GARDEN PEAS (*Pisum sativum*) AND BEANS (*Phaseolus vulgaris*).—Young plants, 10 to 12 cm. high, growing in greenhouse pots, were inoculated on April 16, 1914, by spraying with a spore suspension of the Phoma fungus. The spraying was done with an atomizer on a cloudy day at a room temperature of 78° F. The plants were not covered. In two weeks no spotting had occurred upon either pea or bean leaves.

A second experiment with bean plants was tried similar to the preceding, except that the plants were placed beneath bell jars after the spraying. At the end of 10 days there was no infection.

PEPPER PLANTS (*Capsicum annuum*).—In February, 1914, some pepper plants just coming into blossom were sprayed with the Phoma spore suspension. The plants were kept under bell jars for 10 days. No infection could be seen after a period of three weeks. Other pepper plants were sprayed March 10, 1914, and placed in an infection cage. There was no infection in two weeks' time.

REISOLATION OF THE FUNGUS

FROM PLANTS INOCULATED IN GREENHOUSE.—Spots have been repeatedly produced by means of inoculations upon tomato fruit growing on greenhouse plants, and the Phoma fungus has been plated out from the discolored tissue. The following inoculations are typical of many others. In February, 1914, diseased spots were produced upon green tomato fruit by means of needle-prick inoculations. From tissue cut from the spots the fungus was recovered in agar plates. Diseased spots were produced upon mature fruit growing on greenhouse vines (Pl. III, fig. 2), and the fungous was isolated in pure cultures on February 2, 1914. The leaves of young and of mature tomato plants were diseased as a result of being sprayed with a fungous suspension in January, 1914. From the spotted leaf tissue taken from both young and old leaves the Phoma was isolated by means of agar plate cultures.

FROM PLANTS INOCULATED IN LABORATORY.—Green, partially ripened, and fully matured tomato fruits were picked from vines growing in the greenhouse and brought to the laboratory for inoculation. Upon these tomatoes spots were readily produced in four to seven days by means of needle-prick inoculations of the Phoma, and the fungus was recovered in pure cultures from tissue taken from the spots.

FROM PLANTS INOCULATED IN FIELD.—From tomato fruit, stem, and leaf tissue diseased through inoculation of the Phoma the fungus was obtained in pure culture by means of agar plates (Aug. 19, 1914).

DESCRIPTION OF THE FUNGUS ON ITS HOST

CHARACTER OF SPOTTING

The Phoma fungus produces spots on green and on ripe tomato fruit and on tomato leaves. The fruit, so far as observed, becomes infected only through wounds, while infection of the leaves and stems may be produced without wounding by means of spore spraying. The spots on ripe tomato fruit are black, carbonaceous, definite in outline, and have the surface covered with small black pycnidia. Surrounding the dark tissue of the spot is a watery-looking zone. Frequently the tissue breaks, and a grayish white mycelium develops in the cracks. The spots produced upon green fruit vary from brown to black in color and are membranous, with scattered pycnidia on the surface. Leaves of both young and mature tomato plants are susceptible to spotting. The spots are brown to almost black, definite in outline, and variable in size. Frequently several spots coalesce, forming irregular blotches. The lower leaves became infected first in the experiments, spots appearing upon blade and petiole. Within two weeks after inoculation by spraying, the leaves had become discolored and shriveled and were falling from the stem. Upon the surface of the leaf spots small dark pycnidia occurred singly or in groups. Reisolations in plate culture were made from tissue cut from diseased spots.

MYCELIAL GROWTH

The mycelium of this fungus forms a dense network of hyphal threads within the tissues of the host plant. The hyphæ are septate, frequently branching, and are hyaline to brown in color. Various stages in the development of pycnidia were observed in the tissue of the host, from the first intertwining of hyphal threads to the formation of a definite fruiting chamber, having dark irregularly shaped outer cells. Numerous oil drops were observed in hyphæ grown upon artificial media.

PYCNIDIAL DEVELOPMENT

The pycnidia are subglobose, membranous to carbonaceous, smooth, slightly papillate with a distinct central pore (occasionally two pores), color varying from almost hyaline to black, according to age and medium. There is considerable variation in size. Pycnidia occur scattered or in groups. They are at first covered by the epidermis, but later break through, the spores issuing in long coils through a central pore. In culture the flesh-colored spore exudate frequently forms a slime on the surface of the medium. The pycnidial wall is thin, with brownish outer cells and hyaline inner cells. One-celled pycnospores are developed from the end of the delicate filiform basidia which arise from the inner cells.

Pycnidia from cultural media and from tomato fruit were measured, with the following results:

Corn-meal culture. Average of 10 pycnidia, 151μ, variation 107 to 174μ.
Beef-agar culture. Average of 10 pycnidia, 103μ, variation 59 to 211μ.
Tomato fruit. Average of 40 pycnidia, 139μ, variation 53 to 348μ.

From these measurements it will be seen that an average of 60 measurements gives a diameter of 131μ, with a variation ranging from about 50 to 350μ.

PYCNOSPORES

Only one kind of spore is produced in the pycnidium, the hyaline 1-celled pycnospore. These spores are continuous, 2-guttulate, subcylindrical to subglobose in shape, with blunt, rarely tapering ends, and are produced singly on the unbranched filiform basidia which arise from the inner hyaline cells of the pycnidial wall.

No definite stroma is formed and no perithecial stage has been observed either in culture or upon the host tissue.

Pycnospores were found to vary considerably in size. The following measurements (Table IV) were made of spores grown upon media favorable to their development.

TABLE IV.—*Variation in size of pycnospores of the Phoma fungus*

Source of spores.	Number measured.	Average size.	Variation in size.
		μ	μ
Tomato fruit	40	4.0 by 2.2	3.4 to 8.5 by 1.7 to 3.7
Tomato leaf	20	4.6 by 2.7	3.2 to 6.8 by 1.7 to 3.4
Potato cylinder	10	5.7 by 2.3	3.4 to 8.5 by 1.7 to 3.4
Melilotus stem	10	4.5 by 2.2	3.0 to 5.4 by 1.8 to 3.2
String-bean agar	20	4.7 by 2.6	3.4 to 6.8 by 1.7 to 3.4
Corn-meal culture	40	4.3 by 2.1	3.4 to 6.8 by 1.7 to 3.5

The average size of 140 pycnospores is found from these measurements to be 4.7 by 2.3μ, with a variation of 3 to 8.5 by 1.7 to 3.7μ.

CULTURAL CHARACTERISTICS OF THE FUNGUS

The fungus grows well on all of the media commonly used in culture, good spore development being obtained on corn meal, string-bean agar, oat agar, potato cylinders, and tomato stems. Cultural characteristics of the fungus upon a number of different kinds of media are here given.

BEEF AGAR.—Fungous colonies appear on beef agar (+15) plates within two to three days. These colonies, varying in size from pin points to 5 mm. in diameter, are round, whitish, and somewhat cottony in appearance. The hyphæ branch freely, radiating toward the circumference of the colony. Beef agar is not favorable for spore development, although pycnidia and spores are sometimes produced.

IRISH-POTATO AGAR.—Whitish mycelial growth appears within two to three days. At first cottony in appearance, it later forms a compact mat of hyphal threads. In 5 to 7 days the medium darkens, and in 10 days pycnidia and spores have developed. Pycnidia develop more slowly and less abundantly upon this medium than upon the string-bean, prune, or oat agars.

STRING-BEAN AGAR.—Round whitish fungous colonies appear in two to three days. A few days later the surface growth becomes a compact mat, neutral gray in color, beneath which lies a darker substratum containing the pycnidia. Mycelial growth is more abundant than on beef agar, and the colonies are fuller, with somewhat convex surfaces. Pycnidia form within 6 to 10 days and within 10 to 14 days an exudate may be seen at the apex of the pycnidium, at first in the form of drops and later as a thick slime overspreading the surface of the culture.

LIMA-BEAN AGAR.—A whitish cottony growth appears within three to five days and a few days later pycnidia form, causing a darkening of the substratum. The pycnidia are round to irregular in outline, separate or in clusters, and show the characteristic slimy exudate.

PRUNE AGAR.—In two to three days there is a whitish fungous growth, which a few days later becomes grayish green in color. Single hyphæ when examined under the microscope appear olive green. These hyphæ contain numerous oil drops which disappear when treated with ether. With pycnidial development a dark, almost black color is produced in the substratum, while the spore exudate forms a slime on the surface.

OAT AGAR.—An abundant mycelial growth develops within three to five days. Later this growth darkens as a result of pycnidial development and there is an abundant spore production.

SYNTHETIC AGAR.[1]—A scant mycelial growth develops within three to five days, at first whitish but later becoming neutral gray in color. Spore development is poor.

STERILIZED POTATO CYLINDERS.—Within two to three days a whitish mycelium develops upon the surface of the potato plant. As growth continues, the mycelium becomes compact, varying in color from Ridgway's[2] neutral gray to mouse gray. With the development of pycnidia the substratum darkens and at the end of two weeks the growth becomes dark and feltlike, showing drops of exudate on the surface. Six-weeks-old potato cultures become carbonaceous in appearance and soot-black in color.

STERILIZED COTTON STEMS.—Upon this medium there is scant mycelial growth in four to five days. Later the stem becomes covered with a dark growth consisting of hyphal threads, dark pycnidia, and spore masses.

STERILIZED SWEET-CLOVER STEMS (*Melilotus alba*).—A scant grayish white mycelium develops upon the surface of the stem. Later the sub-

[1] Darwin, Francis, and Acton, E. H. Practical Physiology of Plants. 321 p., illus. Cambridge, England, 1894.

[2] Ridgway, Robert. Color Standards and Color Nomenclature . . . 43 p., 53 col. pl. Washington, D. C., 1912.

stratum darkens and the pycnidia empty their spore contents to the surface.

STERILIZED TOMATO STEMS.—Green tomato stems sterilized in 5 c. c. of distilled water afford a good medium for growth. A whitish cottony mycelium is first seen. This later develops into a dark compact matlike growth which covers the stem and spreads over the surface of the water as a firm pellicle. Within and upon the surface of this mat numerous dark pycnidia develop, and from these the spore masses issue in coils. Microscopical examination of 3-weeks-old tomato-stem cultures show well-developed hyphæ, mature pycnidia, and an abundance of spores. From glycerin slides obtained from these cultures drawings were made showing hyphæ, pycnidia, and spores (Pl. I, figs. 4 and 7). Spores from tomato-stem cultures varied in size from 3.4 to 5.1μ in length and from 1.7 to 2.5μ in width.

STEAMED CORN-MEAL CULTURES.—In sterilized corn-meal flasks (capacity 100 c. c.) growth begins to show in two or three days as a slightly darkened spot. In five days a scant radiating mycelium may be seen and in eight days there is a dark fungous growth 3 to 4 cm. in diameter. Pycnidia are thickly distributed throughout, from the mouths of which slimy spore masses issue in coils. Within two weeks after inoculation the entire surface of the corn meal becomes covered with a dark, compact, slightly greenish mat of fungus 1 to 2 mm. in thickness. The surface of this mat is pimply in appearance, owing to pycnidial development, and is often thrown into folds or depressions within which the exudate from the pycnidia accumulates (Pl. VI. fig. 2). The pycnidia are subglobose and vary in color from hyaline to dark brown and black. Single hyphal strands are brownish and contain numerous oil drops. In microtome sections made of mature pycnidia, fragile threadlike basidia were seen. Upon these basidia the hyaline 1-celled pycnospores are borne. These are subglobular to subcylindrical in shape, 1- to 2-guttulate, and vary considerably in size. In older corn-meal cultures (8 to 10 weeks old) the fungous growth becomes crustlike in composition and soot-black in color, breaking easily when touched with a needle. On the whole the best spore production (with scant mycelium) was obtained upon sterilized corn-meal and tomato-stem cultures. Good mycelial and spore development occurred on oat, string-bean, and lima-bean agar and upon potato cylinders.

Beef agar and synthetic agar proved very poor media for the growth of the fungus.

Characteristic growth upon Melilotus stem, string-bean agar, and potato cylinder is shown in Plate VI, figure 1.

VITALITY OF THE FUNGUS IN CULTURE

The vitality of the Phoma fungus in culture is good. Transfers made from potato cylinders and string-bean agar cultures 6 to 8 months old

showed growth in five days. Under ordinary laboratory conditions stem cultures (tomato, cotton, and sweet clover) dry out in less than a year and the fungus dies. Special medium was used in the hope of obtaining a perithecial stage of the fungus, but no fruiting bodies other than pycnidia have been observed in culture or upon host tissue.

TEMPERATURE RELATIONS

The Phoma fungus grows well at ordinary room temperatures. Transfers were made from a 2-weeks-old corn-meal culture to string-bean agar, potato cylinders, and corn-meal flasks and kept in incubators for a period of 10 days with the results given in Table V.

TABLE V.—*Temperature relations of the Phoma fungus*

Period of growth.	Average temperature (°C.).								
	0.7	1.3	4.9	5.6	7.9	10	14.4	15.6	23.7
10 days....	0	0	0	0	Trace.	Fair.	Good.	Good.	Good.
When removed to room temperature.	Growth within 2 days.	Growth within 2 days.	Growth within 2 days.	Growth within 2 days.	Continued growth.	Continued growth.	Continued growth.	Continued growth.	Continued growth.

Period of growth.	Average temperature (°C.).								
	27.3	27.8	29.5	31.8	32.7	33.1	33.2	33.8	35.8
10 days....	Good.	Abundant.	Abundant.	Trace.	Trace.	0	0	0	0
When removed to room temperature.	Continued growth.	Continued growth.	Continued growth.	Continued growth.	Continued growth.	Growth within 5 days.	Growth within 5 days.	Growth within 5 days.	0

These tests show the minimum temperature of the Phoma fungus to be about 6° C., the optimum temperature about 28°, and the maximum temperature between 32° and 33°.

DISTRIBUTION OF THE DISEASE

Information concerning the distribution of this Phoma disease of tomato has thus far been obtained only through specimens sent in for examination from Cutler, Fla., in 1912; Punta Gorda, Fla.; Cuba; Florence, S. C.; Miamisville, N. Y.; and Herrington, Kans., in 1914.

It seems probable that the disease is widely distributed but because of the presence of associated fungi has often been looked upon as one of the more commonly known fruit-spots. The seriousness of the disease under favorable climatic conditions is suggested in the report of Mr. James Brown, already mentioned, that this fruit-spotting had caused heavy loss among the farmers of Dade County, Fla., in 1912.

TAXONOMY OF THE FUNGUS

The earliest mention of a Phoma fungus as parasitic on tomato fruit is given by Plowright (1881). This fungus associated with three others is given as the cause of a "black spot" upon the crown of the fruit, and is described as follows:

Phoma destructiva. Perithecia carbonaceous, minute, globose, spherical clustered spores, hyaline, oval, cylindrical, binucleate, 5–6 mk. long, by 1.5–1 wide.

Spegazzini (1881, pp. 67–68) published a description of *Phyllosticta hortorum*, a fungus which he found parasitic on the leaves of the eggplant, and Ellis and Everhart (1900) state that it is common on tomato leaves. That there has been considerable confusion in regard to the identity of *Phyllosticta hortorum* (Speg.) is brought out in a recent article by Harter (1914), who sums up the situation in these words (p. 337):

If, therefore, any value is to be given to a comparison made by an author with his own type specimens, it is safe to conclude that *Phyllosticta hortorum* has not yet been found in this country.

In view of this statement, it is doubtful whether the eggplant fungus reported on tomato leaves was *Phyllosticta hortorum* (Speg.).

Cooke (1885, p. 94) thus describes *Phoma lycopersici* (*Phoma herbarum* West.):

Caulicolus. Perithecia punctiform, black, densely gregarious, at first covered by the cuticle, ultimately more or less exposed. Sporules lanceolate, binucleate (0.012 × 0.004 mm.). On stems of tomato.

No mention is made of the occurrence of this fungus upon the fruit, and the size of spores as given is about twice the average size of those of the Phoma described in this paper.

Peck (1887, p. 57) gives the following description of *Phyllosticta lycopersici* as a parasite upon tomato fruit:

Spots large, suborbicular, cinereous; perithecia minute, brown or blackish, opening by a single or sometimes by two pores; spores abundant, oblong or elliptical, .00025 to .0003 inch long, .0001 to .00012 broad. Fruit of tomato.

Except for slight differences in size and shape of spores, this description is similar to that given by Plowright for *Phoma destructiva*.

Marchal (1900) mentions a Phoma as the cause of a new disease of greenhouse-grown tomatoes, but gives no specific name to his fungus.

In order to determine if possible something more definite in regard to the relation between *Phoma destructiva* Plowr. and *Phyllosticta lycopersici* Pk., a type specimen of Peck's fungus was requested and kindly furnished by him from the herbarium of the New York State Museum in October, 1914. This specimen (dated July, 1886) consisted of a small piece of tomato fruit showing a portion of a diseased spot, the tissue of which varied from Ridgway's buff [1] to olive brown in color. On account of the age of the specimen and the presence of a foreign fungus in the tissue,

[1] Ridgway, Robert. Op. cit.

identification of the causal organism was difficult, but in slides made from different parts of the discolored tissue some brownish subglobose pycnidia and a few hyaline 1-celled spores of *Phyllosticta lycopersici* were observed. Efforts made to secure cultures of Phyllosticta from the type specimen were unsuccessful. From descriptions of the fungi given by Plowright and Peck and from examination of the type specimen just described in the opinion of the writer it appears that *Phoma destructiva* Plowr. and *Phyllosticta lycopersici* Pk. are synonymous and that the Phoma described in this article is identical with them. Because of these facts and in order to avoid confusion of names, it has been thought best to adopt Plowright's nomenclature. The fungus described in this paper as causing fruit-rot, stem- and leaf-blight of the tomato is therefore designated as *Phoma destructiva* Plowr. emend.

Phoma destructiva Plowr. emend.

Phoma destructiva Plowr., 1881, *in* Gard. Chron., n. s. v. 16, no. 411, p. 620.
Phyllosticta lycopersici Pk., 1887, *in* 40th Ann. Rpt. N. Y. State Mus. Nat. Hist., 1886, p. 57.
Phoma sp. Marchal, 1900, *in* Bul. Agr. [Brussels], t. 16, no. 1, p. 18.

Diagnosis.—Spots on tomato fruit, brown to black, membranous or carbonaceous, definite. Pycnidia scattered to aggregate, most abundant toward center of spot, subcutaneous later erumpent, glabrous, brownish black, subglobose, slightly papillate, not beaked, ostiolate (usually one, sometimes two pores), 30 to 350μ in diameter; pycnidial wall thin, outer cells brown, inner cells hyaline; delicate filiform basidia arising from inner cells. Pycnospores issue in coils through ostiolum, forming a slimy flesh-colored exudate; hyaline, continuous, 1-celled, 2-guttulate, subcylindrical to subglobose, rarely tapering, variation (100 measurements) 2.8 to 8.5 by 1.7 to 3.4μ; produced singly on unbranched filiform basidia. Hyphæ septate, branching, hyaline to brownish, vacuolate. No definite stroma. No perithecial stage observed.

Habitat.—Parasitic on fruit of *Lycopersicon esculentum*, spots occurring on tomatoes (green and ripe), usually near stem end, 1 to 3 cm. Conspicuous, dark, definite, sometimes coalescing; tissue membranous to carbonaceous, depressed, with dark, minute, pimple-like pycnidia. Observed upon tomato fruit received from Florida, South Carolina, Kansas, New York, and Cuba.

CONCLUSIONS

As a result of inoculation experiments, *Phoma destructiva* Plowr. emend. has been proved an active wound parasite upon green and ripe tomato fruit.

This fungus also causes a leaf spotting of tomato and potato plants.

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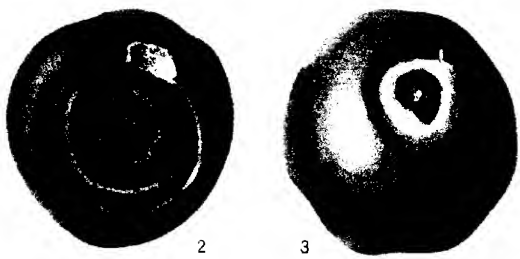
PLATE A

Tomato fruit spotted with *Phoma destructiva*.

Fig. 1.—Natural infection on tomato received from Cutler, Fla., March, 1912.

Figs. 2, 3, 4, and 5.—Spots produced as the result of needle-prick inoculations with *Phoma destructiva*.

Painted by J. Marion Shull.



2 3

4 5


2
3

PLATE B

Potato and tomato leaflets spotted as result of inoculation with *Phoma destructiva*.

Fig. 1.—Potato leaflet from sprayed plant.
Figs. 2 and 3.—Tomato leaflets from sprayed plant.
Painted by J. Marion Shull.

PLATE I

Fig. 1.—*Phoma destructiva:* Group of pycnidia, with surrounding mycelium, showing relation of the parasite to the host tissue. *P*, Pycnidia; *M*, mycelium; *C*, cell of host. Drawn by J. F. Brewer.

Fig. 2.—*Phoma destructiva:* Single hyphæ, showing septation and branching. Drawn by J. F. Brewer.

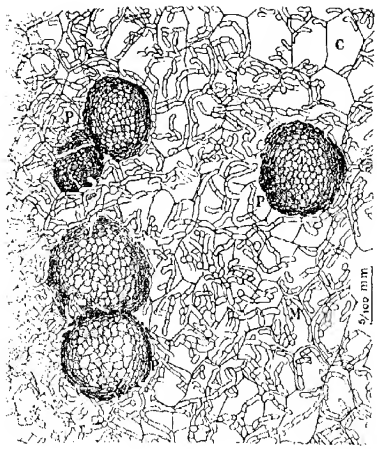
Fig. 3.—*Phoma destructiva:* A few pycnospores highly magnified. Drawn by J. F. Brewer.

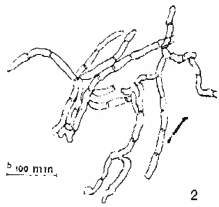
Fig. 4.—*Phoma destructiva:* Pycnidium and surrounding mycelium. Side view, showing spores issuing from ostiolum. Drawn by J. F. Brewer.

Fig. 5.—*Phoma destructiva:* Pycnidium as seen in cross section of diseased tissue of tomato fruit. Mass of pycnospores are within pycnidial chamber. Surrounding the pycnidium are cells of host tissue and cut hyphal threads. Drawn by J. F. Brewer.

Fig. 6.—*Phoma destructiva:* Pycnidium from artificial culture. Cross section showing cell structure of wall, basidia, and pycnospores. Semidiagrammatic drawing by J. F. Brewer.

Fig. 7. —*Phoma destructiva:* Pycnidium and surrounding mycelium. View from above, showing ostiolum. Drawn by J. F. Brewer.


C
P
P
M
5/100 mm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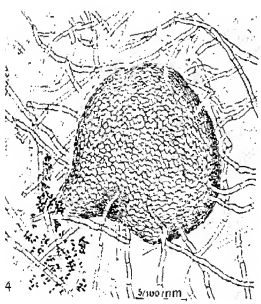
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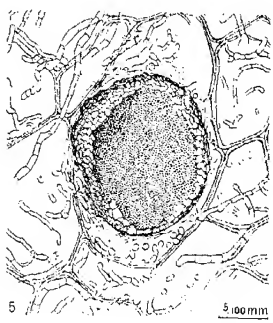
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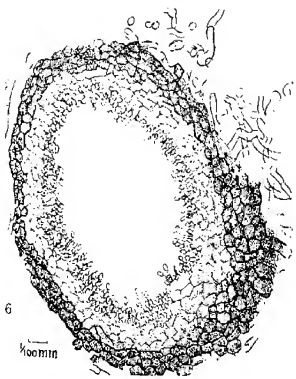
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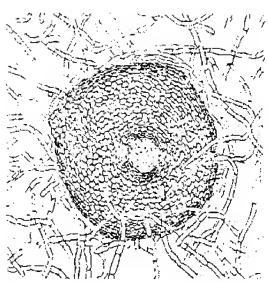
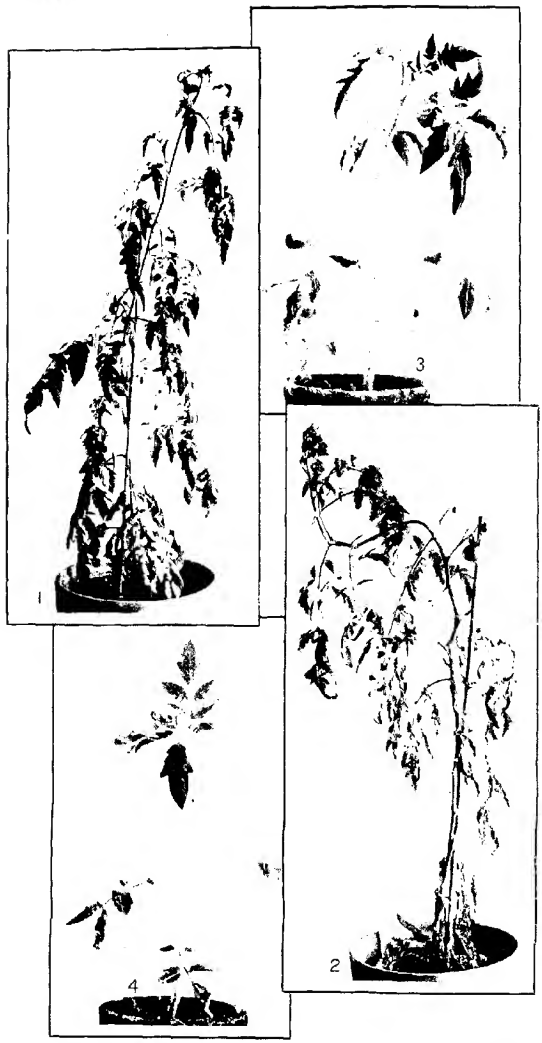
1/100 mm




1
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3
4

PLATE II

Mature and young tomato plants grown in greenhouse, showing infection by *Phoma destructiva*.

Figs. 1 and 2.—Mature plants. Fig. 1.—Control plant. Fig. 2.—Diseased plant, 10 days after spraying with a spore suspension of *Phoma destructiva*. Foliage spotted and lower leaves fallen from stem.

Figs. 3 and 4.—Young plants with foliage spotted as a result of inoculation with *Phoma destructiva*. Photographed eight days after inoculation.

PLATE III

Fig. 1.—*Phoma destructiva:* Natural infection on tomato fruit received from Cuba.

Fig. 2.—*Phoma destructiva:* One tomato fruit on vine diseased by needle-prick inoculation. Other fruit not inoculated showed no infection.

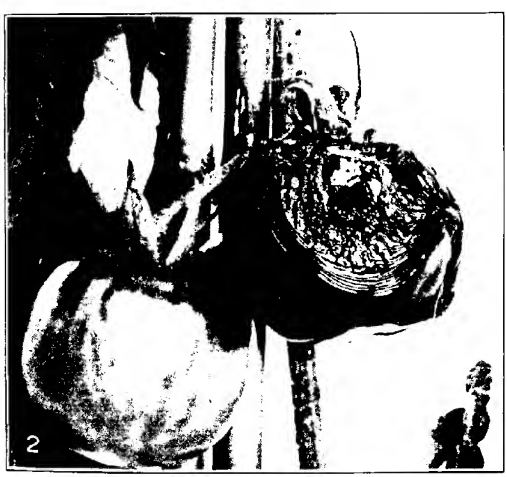
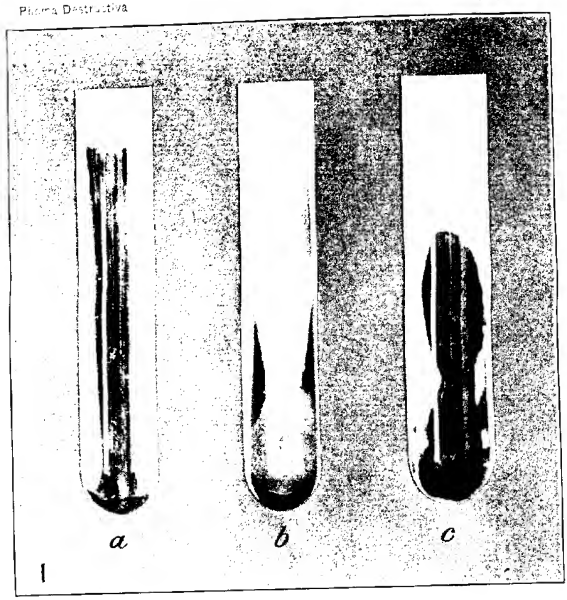

2

PLATE IV

Tomato leaves showing spots produced by spraying with a suspension of *Phoma destructiva*. Photographed 12 days after treatment.

PLATE V

Potato leaves affected with spots produced by spraying with a suspension of *Phoma destructiva*. Photographed 10 days after treatment.


a
b
c
1

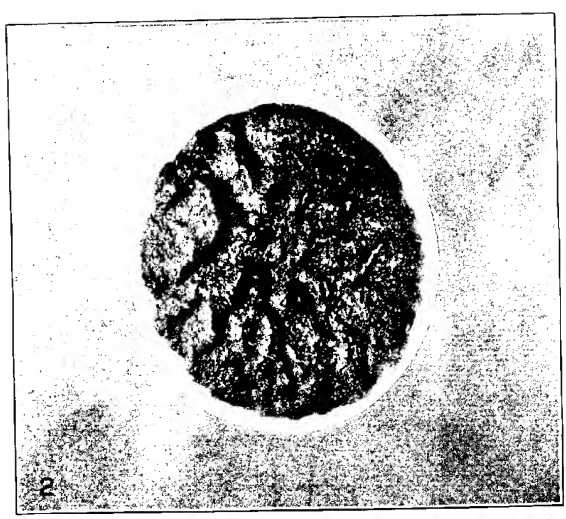

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PLATE VI

Phoma destructiva: Artificial cultures.

Fig. 1. Test tube cultures. *a*, Melilotus stem; *b*, string-bean agar; *c*, potato slant.
Fig. 2.—Corn-meal flask culture.

AVAILABILITY OF THE NITROGEN IN PACIFIC COAST KELPS

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PURPOSE OF THE STUDY

One of the first problems to be solved in the study of the fertilizing value of the three major kelps of the Pacific coast, *Macrocystis pyrifera*, *Nereocystis luetkeana*, and *Pelagophycus porra*, is the form in which these plants can most economically and completely be utilized. The difficulties inherent in any plan for manufacturing pure potash salts as their sole product have already been pointed out by J. S. Burd (5),[1] of the California Agricultural Experiment Station. It has also been shown in the same publication that the most feasible plan of manufacture so far suggested is to dry and grind the kelp and to use it either alone or preferably in combination with other ingredients as the basis for a mixed fertilizer. The advantages of such a method of operation are at once obvious. The low cost of operation and the probable complete utilization of the plant food present are among the most evident. In the latter connection, however, it at once becomes very pertinent to inquire what will be the probable fate of the organic matter of the kelp when added to the soil.

Will it readily decompose and aid in the formation of humus? Will its nitrogen and phosphoric acid become freely available for the crops upon which it is used?

In addition, it will be very desirable to know what effect not only the organic matter of the kelp but also its salts will have upon the biological, chemical, and physical processes of the soil and also upon the plants growing therein. The laboratory studies taken up in this paper are intended to show the availability of the organic matter of the kelps studied and also to give some insight into the effect of the salts present in them upon the biological activities of the soil.

The study of the changes taking place in organic matter when added to the soil is of the most absorbing interest to modern investigators. It has been the subject of many excellent researches during recent years, though earlier work dates back to the time of Boussingault (2) in 1834. At present the most satisfactory procedure for studying this problem is undoubtedly the so-called beaker method of soil bacteriology, which measures the availability of a material by the readiness with which it will ammonify and nitrify when a small portion is added to a definite quantity of soil. This method is now so well known that it hardly

[1] Reference is made by number to "Literature cited," pp. 37–38.

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needs further description, and the reliability of the results obtained by it has been fully demonstrated through the work of a large number of investigators who have taken it up since it was first proposed by Stevens and Withers (18, 19, 20). Lipman and Brown (11), working together at the New Jersey Experiment Station, have proposed the same manner of experimentation, and this method of investigation has been almost universally adopted. The same general procedure was therefore considered to be suitable for the study of the availability of the organic matter of the kelps in question.

EXPERIMENTAL METHODS

In order that the results should be comparable to those obtained under field conditions, the soils used throughout all the series were fresh field soils. The surface soil was removed to a depth of 1½ to 2 inches, it having been shown by Löhnis (15, p. 571) and others that this portion does not possess a typical bacterial flora. A large sample was then taken from the next 3 or 4 inches of soil, carried immediately to the laboratory, spread out in a darkened room, and dried to a point where it could be readily sifted and thoroughly mixed. In this condition it was still moist and, according to the work of Brown (3, 4), undoubtedly possessed a bacterial flora which was representative of field conditions. Two-hundred-gram portions of this soil were then placed in sterile tumblers, which were covered with Petri dishes. The various amounts of material to be investigated were then added, the whole being thoroughly mixed. Sterile distilled water was added to make optimum moisture conditions, which in most cases were found to be about 18 per cent. The samples and duplicate blanks were then incubated at 28° to 30° C. for the required time. At the end of the period they were transferred to copper distilling flasks. About 400 c. c. of distilled water were added, together with magnesium oxid and a small quantity of paraffin to prevent foaming. The ammonia present was distilled into standard *N*/10 hydrochloric acid and the excess portion titrated with standard *N*/10 ammonia in the usual manner.

Upon consideration of the analytical results obtained from the first portion of Series I and the very slow rate of ammonification of *Macrocystis pyrifera* revealed by these results, it seemed desirable to have determinations of the amount of nitrification which was probably taking place concurrently. This would, it was felt, be a satisfactory check on the progress of the transformation of the nitrogen through the cycle of changes taking place in the soil. The residues remaining in the copper distilling flasks after the determination of ammonia were immediately transferred to 1-liter volumetric flasks of the Giles pattern, or, where much small gravel was present, to 1-liter graduated cylinders. They were then made up to 1 liter and sufficient additional distilled water added to compensate for the displacement caused by the soil and ferti-

lizing material present, this figure being determined for each set on other duplicate portions. The whole solution was thoroughly mixed and an aliquot portion taken for the determination of nitrates by the aluminum-reduction method. The essential procedure as outlined by the American Public Health Association (1, p. 25) was followed. Previous work with this method on sewage samples, which were solutions of essentially the same type, had shown it to be very satisfactory for nitrate determinations. Burgess (6) has also found this method excellently adapted to the determination of nitrates in soils. The accuracy of the above procedure was also checked by running several series of triplicate determinations in which 200 gm. of soil were taken and distilled with magnesium oxid, as shown above, and the nitrates in the residue determined. To other triplicate sets varying quantities of potassium nitrate were added. They were then similarly analyzed. The agreement throughout was excellent. In some cases it was absolute. In none did the divergence exceed the ordinary analytical variation.

SERIES I.—COMPARATIVE AMMONIFICATION AND NITRIFICATION OF DRIED KELP, DRIED BLOOD, AND COTTONSEED MEAL, USING CLAY ADOBE SOIL.

In the first series *Macrocystis pyrifera*, *Nereocystis luetkeana*, and *Pelagophycus porra* were each used in the proportion of 10 gm. of dried and ground kelp to 200 gm. of fresh field soil. This soil was taken from the campus botanical gardens and was in texture a slightly modified clay adobe. In order to compare the kelp with substances of well-known availability, portions of 1 gm. of dried blood and 2 gm. of cottonseed meal were also added to duplicate sets of tumblers, while blank determinations of untreated soil were started to determine the ammonia and nitrate production occurring in the natural soil. The kelp employed was in each case a composite made up from the analytical samples of that variety. These had been dried to a constant weight at 100° to 105° C. for a period of three to nine hours and then ground so as to pass through a 0.5-mm. sieve. The percentage composition of the kelp was as follows:

Species of kelp.	Moisture.	Nitrogen.	Total soluble salts.
Macrocystis pyrifera	4.71	1.07	30.77
Nereocystis luetkeana	5.24	1.66	46.83
Pelagophycus porra	4.36	1.22	45.64

It will be noted that the moisture content was fairly uniform in all varieties, while both the *N. luetkeana* and *P. porra*, as usual, contained larger quantities of nitrogen and total soluble salts than the *M. pyrifera*. The composition of the salts, both soluble and insoluble, added to the soil when applying kelp will be of considerable interest in this connection. It was impossible to make complete ash analyses of all the kelps used throughout these studies. Mr. P. L. Hibbard, of this laboratory, however, has made analyses of representative plants of each species, and it is

felt that these will be fairly typical of the materials used in this investigation. The figures as quoted are expressed as the percentages of actual radicles occurring in the ash, and the total percentage of ash is the amount found in the plants when calculated to a moisture-free basis.

Composition of the ash of the harvestable portions of Macrocystis pyrifera, Nereocystis luetkeana, and Pelagophycus porra

Constituent.	*Macrocystis pyrifera.*	*Nereocystis luetkeana.*	*Pelagophycus porra.*
	Per cent.	*Per cent.*	*Per cent.*
Ca	4.96	2.10	2.09
Mg	2.24	1.55	1.71
Na	10.52	11.05	8.63
K	29.46	32.66	34.73
Fe_2O_3	.43	.17	.26
Al_2O_3			
Cl	34.93	40.89	40.83
SO_4	7.92	4.63	4.84
CO_2	4.44	3.10	1.66
PO_4	2.30	1.91	2.18
Total	97.20	98.06	96.93
Total percentage of ash in water-free material	35.62	50.57	52.66

There are several very important deductions to be made from these analyses when considering them from the point of view of soil fertility. The very large percentage of total ash present is at once apparent; and from preceding determinations of the soluble salts present in similar samples it is evident that the larger portion of the total ash, about 85 per cent, is, in fact, water-soluble.

Some quantitative separations of the soluble salts were made which showed that small quantities of the calcium and traces of the magnesium only were dissolved, while all the potassium and chlorin and the major portion of the sodium and sulphate ions went into solution.

The immediate effect, then, of the incorporation of kelp in the soil is a very considerable addition of soluble salts. Three-fourths or more of these will be potassium chlorid or sulphate, while the other fourth will largely consist of sodium chlorid and sulphate. The potash will be fixed in the soil by its well-known precipitative power for this compound, while the sodium salts will in any ordinarily well-drained soil be carried off in the drainage water. As the kelp gradually decomposes, the compounds present in the soil will approach those represented in the complete ash analyses above given. They will then tend to make a much more balanced solution, even if all the sodium salts be not removed by that time.

From C. B. Lipman's work on the toxicity and antagonism of various salts on soil bacteria both in solutions and in soil (7, 8, 9, 10, 12) it would be reasonable to expect that if any disturbance of the biological activities of the soil is caused by the soluble salts from applications of kelp this influence would be very likely to decrease as the soil solution

becomes more balanced in its character. He has shown that the principles of antagonism previously established by Loeb (13, 14) and Osterhout (16, 17) for animals and plants also hold in general for soil bacteria.

Considering now the results obtained in Series I, it will be observed that the first ammonia determinations were made at the end of 9 days' incubation. Other duplicate sets were analyzed at periods of 11, 15, 19, 48, and 102 days. Even at the end of 9 days it will be seen that very striking differences exist. As was to be expected, the dried blood ammonifies most readily. Next in rate of conversion is the cottonseed meal, while *Nereocystis luetkeana* is not very far behind this. *Pelagophycus porra* is distinctly slower, while *Macrocystis pyrifera* shows only a trace converted.

Still considering the ammonification, the same relationship holds with the kelps throughout the whole series. After 15 days the major portion of the nitrogen, which was changed to ammonia with the blood and the cottonseed meal, has been converted over into nitrates. It must be pointed out in the discussion of the results of this first series that the amounts of kelp added were quite excessive. The only reason for employing such quantities even in a laboratory study was that it furnished an amount of nitrogen which would give a very satisfactory analytical figure should conversion readily take place. It had been supposed also that all the kelps were probably very available and would decompose readily, so the large quantities used were intended to test this belief under extreme conditions.

Taking this series as a whole, it will be seen that even with the large content of soluble salts furnished by the kelp, ammonification took place with surprising readiness in the case of Nereocystis and Pelagophycus, while with Macrocystis it was only after 48 days had elapsed that definite small amounts were converted to ammonia. Nitrification, however, in the case of all the kelps was almost entirely inhibited; in fact, even the amounts originally present in the soil did not remain at the end of the period in the form of nitrates. At the close of the final period, 102 days, duplicate sets of tumblers were mixed and the total amount of nitrogen determined by the modified Kjeldahl method to include the nitrogen of nitrates.

Material.	Recovered. *Mg.*	Added. *Mg.*
Macrocystis pyrifera	100. 5 100. 5	107
Nereocystis luetkeana	165. 7 162. 3	166
Pelagophycus porra	114. 5 125. 9	122
Dried blood	100. 8 92. 2	132
Cottonseed meal	124. 7	128

There has apparently been a slight denitrifiation in the case of the dried blood, but with the other materials the amount recovered is substantially the same as that which had been added.

TABLE I.—*Series I.* *Comparative ammonification and nitrification of dried kelp, dried blood, and cottonseed meal*

[Soil used: Clay adobe from the campus botanical gardens, Berkeley, Cal.]

Material added to 100 gm. of soil.	Quantity of material added.	Quantity of nitrogen added.	Quantity of soluble salts in kelp added.	Incubated 9 days.	Incubated 11 days.			Incubated 15 days.			Incubated 19 days.			Incubated 48 days.			Incubated 60 days.			Incubated 102 days.		
				Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.
	Gm.	*Mg.*	*Gm.*	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*	
Macrocystis pyrifera	10	107	3.08	0.34	−0.07	−0.95	None.	−0.35	−1.57	None.	0.98	−1.50	None.	1.96	−4.24	None.	2.31	−4.60	None.	2.87	−7.35	None.
				.55	.49	−.95		.29	−1.52		.35	−1.50		2.66	−4.50		4.56	−4.64		2.45	−7.30	
Nereocystis luetkeana	10	166	4.68	23.30	27.61	1.75	18.75	46.30	.18	22.91	47.83	.20	28.55	51.56	−4.50	31.90	61.29	−3.20	34.99	62.28	−5.43	33.92
				19.25	33.81	−.90		30.90	−1.32		46.33	.45		62.76	−3.80		61.17	−3.08		60.32	−4.55	
Pelagophycus porra	10	222	4.56	9.47	9.47	−.95	6.90	14.32	−1.37	11.07	16.47	−1.20	12.11	23.26	−4.60	13.31	21.37	−4.20	14.34	22.91	Lost.	18.74
				6.95	9.19	−.85		15.44	−1.37		15.61	−1.30		18.22	−4.40		22.21	−4.40		27.67	−6.55	
Dried blood	1	132	.....	44.32	36.71	4.75	32.54	32.20	46.33	51.19	26.03	64.80	54.77	11.49	65.20	61.94	9.32	75.00	63.32	13.10	53.25	48.77
				44.58	39.71	4.75		33.28	23.33		23.61	30.20		11.63	75.20		11.84	71.00		10.16	52.25	
Cottonseed meal	2	128	.....	30.28	27.31	2.75	23.56	20.36	13.33	27.67	16.74	30.20	34.62	1.68	71.20	55.63	3.01	53.00	44.54	3.85	50.25	46.34
				31.12	26.61	3.75		18.82	18.33		15.48	26.20		4.35	65.20		3.01	55.00		4.28	60.25	

SERIES II.—COMPARATIVE AMMONIFICATION AND NITRIFICATION ON FOUR TYPICAL SOILS OF FRESH AND DRIED MACROCYSTIS, COTTONSEED MEAL, AND DRIED BLOOD

The results obtained in Series I clearly show the readiness with which *Nereocystis luetkeana* and *Pelagophycus porra* are decomposed in the soil with the formation of ammonia. With smaller concentrations of kelp, this would probably be readily converted over to nitrates. These varieties, however, are only of minor importance from a commercial standpoint. As has been pointed out by J. S. Burd (5), the Macrocystis is the only one which can be considered commercially important in California.

A second series of ammonification and nitrification studies was therefore started to further investigate the availability of *Macrocystis pyrifera*. It was felt also that the composite analytical sample of Macrocystis which was used in the first series had possibly been dried for a longer time than would be the case if the kelp were handled commercially.

In the second series, therefore, *M. pyrifera*, which, after having been thoroughly sun-dried, contained 10.52 per cent of moisture, 0.80 per cent of nitrogen, and 30.80 per cent of soluble salts, was employed. Fresh kelp, just gathered from the ocean, was also obtained at Pacific Grove, Cal., brought to Berkeley the same day, and placed in a refrigerator till it was used the following morning. The fresh kelp contained 87.9 per cent of moisture, 0.22 per cent of nitrogen, and 4.60 per cent of soluble salts. These two forms of kelp were again contrasted with dried blood and cottonseed meal. In order to study the effect on a variety of soils, four different types of fresh field soil were employed. The first was again the clay adobe from the university campus. The next was a highly productive alluvial loam from Hayward, Cal. A moderately productive clay loam was also obtained from Hayward, and for the fourth a light sandy soil from the vegetable-garden district of South San Francisco.

The series was divided into two sets. In the first set both the fresh and the dry Macrocystis were partially leached with distilled water. Any such treatment of the fresh kelp would be impracticable on a large scale. The thick colloidal solutions formed are extremely difficult to pour or filter. A great deal of moisture was also retained by the kelp. After leaching, the fresh sample contained 95.46 per cent of moisture, 0.09 per cent of nitrogen, and 1.46 per cent of soluble salts. The dried and ground Macrocystis was easily treated with about 15 c. c. of distilled water. This removed a few milligrams of nitrogen and two-thirds of the soluble salts. The tumblers of this set were incubated for 7 days.

In the other set the kelp was added to the soil without leaching, and the period of incubation was 11 days. The fresh kelp used in both sets was ground by passing it through a food chopper. The dry Macrocystis was ground, as in Series I, and the same analytical procedure was followed. (See Table II.)

TABLE II.—*Series II. Comparative ammonification and nitrification on four typical soils of fresh and of dried Macrocystis pyrifera, leached with distilled water and unleached, as contrasted with cottonseed meal and dried blood*

Period of incubation and material added to 200 gm. of soil.	Quantity of material added.	Quantity of nitrogen added.	Quantity of soluble salts in kelp added.	Clay adobe soil. Berkeley.			Highly productive loam from Hayward.			Moderately productive loam from Hayward			Sandy soil from San Francisco.		
				Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.
INCUBATED 7 DAYS:	*Gm.*	*Mg.*	*Gm.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*	
Fresh *Macrocystis pyrifera*, leached	35	33.00	0.50	2.45 2.45	1.30 Lost.	11.36	+ 0.49 .21	— 2.00 .00	None.	0.21 .63	4.40 1.20	9.76	2.03 2.45	0.65 .85	9.00
Dry *Macrocystis pyrifera*, leached	5	32.36	.51	.91 .63	— .59 Lost.	.72	.49 Lost.	— 3.00 — 3.00	None.	.35 .21	— 1.86 — 1.86	None.	— .07 .21	— .45 — .47	None.
Dried blood	1	132.00	.....	52.75 50.05	3.80 3.30	41.85	73.01 72.84	8.80 17.80	19.48	47.84 45.32	2.50 4.00	37.75	56.25 59.11	2.25 2.45	44.34
Cottonseed meal	2	128.00	.....	46.28 39.86	1.30 1.10	32.74	6.94 7.36	13.80 12.80	15.98	42.39 42.39	— 1.30 .00	32.61	44.48 43.50	.25 — .25	34.37
INCUBATED 11 DAYS:															
Fresh *Macrocystis pyrifera*, unleached	35	77.00	1.63	9.04 8.62	1.00 1.00	12.44	.98 .42	3.20 3.20	4.93	13.29 14.42	5.96 6.56	25.44	15.12 14.02	— .65 — .65	17.61
Dry *Macrocystis pyrifera*, unleached	5	40.00	1.54	— 1.33 — 1.47	— .70 — .60	None.	.56 — .56	— 4.60 — 4.00	None.	— .17 — .17	— 2.88 — 2.84	None.	.14 .02	— .53 — .73	None.
INCUBATED 9 DAYS:															
Dried blood	1	132.00	.....	46.59 46.45	15.00 14.20	46.30	2.10 1.54	57.20 55.20	43.95	45.64 45.50	16.96 18.96	48.13	57.72 56.46	12.85 6.05	50.10
Cottonseed meal	2	128.00	.....	36.68 36.92	8.60 8.00	35.00	.28 1.12	40.20 40.20	31.95	39.90 39.90	12.96 16.96	42.86	44.97 44.97	2.55 4.05	37.71

The analytical tables furnish some interesting results. It is at once evident that the fresh Macrocystis, whether leached or unleached, is readily decomposed by practically all of the four soils. It is also apparent that the leaching, which has removed a considerable portion of the soluble salts present, has not increased the rate at which the nitrogen is changed to more available forms. It was originally planned to incubate all of this series for 7 days. Upon analyzing the cultures with leached kelp, however, it was found that the decomposition had not been so rapid as anticipated, and it was supposed that the unleached samples would be slower still. They were therefore incubated for 11 days. The result proves that decomposition had been going on in this portion of the set as rapidly as in the leached. The removal of the salts from Macrocystis would not, therefore, be advantageous to increase its rate of decomposition. It is also interesting to note that nitrification has taken place with the fresh kelp in all of the soils except the sandy, a type which is frequently somewhat slow in this respect.

With the dry Macrocystis in this series both the leached and unleached gave practically a negative result. It was to be expected that there would be some disparity in the availability of the fresh and dried material. A green-manuring crop which is dried before it is turned under the soil differs somewhat similarly from the same crop turned under in a green condition, the fresh legumes decomposing more readily than the dried. The difference between the fresh and the dry Macrocystis is, however, very great. We have no proof as yet of the final availability of the dry Macrocystis. To demonstrate this, we require a positive gain of both ammonia and nitrates. It is of decided importance in our study to have found that the fresh Macrocystis will decompose with any average soil.

SERIES III.—THE EFFECT OF LARGE AND SMALL AMOUNTS OF DRIED MACROCYSTIS AND NEREOCYSTIS ON THE AMMONIFICATION AND NITRIFICATION OF DRIED BLOOD

The results so far obtained have left several points undecided. It has been indicated that oven-dry Macrocystis decomposes quite slowly, while Nereocystis at least ammonifies readily. Fresh Macrocystis both ammonifies and nitrifies. It now becomes desirable to know how long a period will elapse before dry Macrocystis in small or large quantities will decompose. Will dry Nereocystis in moderate amounts nitrify, as well as ammonify? And as kelp will probably be used with other organic fertilizers, how will materials like blood or tankage behave when the salts of kelp are also present?

The following series was therefore planned: Air-dried *Macrocystis pyrifera* and oven-dried *Nereocystis luetkeana* were each added to duplicate sets of tumblers. The amounts used furnished 10, 50, and 100 mg. of nitrogen. To another set of tumblers with the same three quantities of kelp, dried blood to furnish 10 mg. of nitrogen was added. In like

manner dried blood to furnish 100 mg. of nitrogen was added to other duplicate sets of kelp and soil. The above combinations were contrasted with duplicates containing 10 mg. and 100 mg. of nitrogen from dried blood, and also with tumblers of untreated soil.

The Macrocystis used contained 8.47 per cent of moisture, 0.90 per cent of nitrogen, and 35.68 per cent of soluble salts. The composition of the Nereocystis was 3.51 per cent of moisture, 1.70 per cent of nitrogen, and 46.47 per cent of soluble salts. The dried blood was the same as in Series I and II. The fresh field soil employed was the clay adobe from the campus botanical gardens. The ability to ammonify and nitrify which was shown by this soil in Series II was considered thoroughly satisfactory. It could be obtained readily in a fresh condition and for these reasons was used in this and all further work.

Several duplicate sets of tumblers with additions of Macrocystis and blood were prepared. These were incubated for periods of 2, 4, 11, and 15 weeks. Two sets of Nereocystis were incubated for 2- and 4-week periods, respectively. (See Tables III and IV.)

In Tables III and IV the total number of milligrams of nitrogen found as ammonia and as nitrates has been given, as well as the number of milligrams gained. This has been done because these data in some cases give more information than those recorded under the head of gain.

The results where the Macrocystis is the only addition to the soil will first be considered. The tumblers with 10 mg. of added nitrogen from kelp have apparently too small an amount present to yield any definite result. The variations in each period between the treated tumblers and the untreated soil are insignificant. The addition of 50 mg. of nitrogen in Macrocystis produces some striking changes. At the end of 2 and 4 weeks the result is the same; a small gain of ammonia has taken place. This is accompanied by a great reduction in the quantity of nitrates present. At 11 weeks there is not only an appreciable gain of ammonia, but the nitrate production is almost normal. At the end of 15 weeks the ammonia present is the same as in the blank, and 6 per cent of nitrogen has been gained in nitrates. This is considered to be an appreciable figure, especially in view of the improved nitrate content shown in the previous set. The results from the tumblers with 100 mg. of added Macrocystis nitrogen corroborate these from the 50 mg. portions. The 2- and 4-week determinations show a trifling gain of ammonia and considerable losses of nitrates. At 11 weeks there is an appreciable gain of ammonia, which is larger at the end of 15 weeks. The nitrate content remains low throughout all the sets, just as it did in Series I. It is, however, considered to be significant that with both of the larger amounts of kelp a distinct ammonification appeared at the same period, 11 weeks. This kelp had not been dried completely, as had that in Series I, a fact which has apparently considerable bearing on the availability of Macrocystis.

TABLE III.—*Series III, part 1. Effect of small and large amounts of dried Macrocystis pyrifera on the rate of ammonification and nitrification of dried blood in clay adobe soil from Berkeley, Cal.*

Material added to 200 gm. of soil.	Quantity of kelp added to 200 gm. soil.	Quantity of nitrogen added.	Quantity of soluble salts in kelp added.	Incubated 2 weeks.					Incubated 4 weeks.					Incubated 11 weeks.					Incubated 15 weeks.				
				Total quantity of nitrogen present in ammonia.	Average quantity of nitrogen gained in ammonia.	Total quantity of nitrogen present in nitrates.	Average quantity of nitrogen gained in nitrates.	Average percentage of nitrogen in blood changed to ammonia and nitrates.	Total quantity of nitrogen present in ammonia.	Average quantity of nitrogen gained in ammonia.	Total quantity of nitrogen present in nitrates.	Average quantity of nitrogen gained in nitrates.	Average percentage of nitrogen in blood changed to ammonia and nitrates.	Total quantity of nitrogen present in ammonia.	Average quantity of nitrogen gained in ammonia.	Total quantity of nitrogen present in nitrates.	Average quantity of nitrogen gained in nitrates.	Average percentage of nitrogen in blood changed to ammonia and nitrates.	Total quantity of nitrogen present in ammonia.	Average quantity of nitrogen gained in ammonia.	Total quantity of nitrogen present in nitrates.	Average quantity of nitrogen gained in nitrates.	Average percentage of nitrogen in blood changed to ammonia and nitrates.
	Gm.	*Mg.*	*Gm.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Untreated soil	.....	.....	.....	1.54 1.82	.....	4.20 4.60	.....	.....	1.82 1.82	.....	8.00 7.00	.....	.....	2.66 2.38	.....	11.00 10.00	.....	.....	2.94 2.66	.....	12.00 12.00	.....	.....
Dried blood	.....	10	.....	2.94 1.82	0.70	12.00 12.00	7.60	83.00	1.82 1.68	−0.07	14.00 15.00	7.00	69.30	2.52 2.80	0.14	15.00 15.00	4.50	46.40	2.24 2.66	−.35	20.00 20.00	8.00	76.50
Do.	.....	100	.....	16.25 16.95	14.92	52.00 52.00	47.60	62.52	2.52 2.80	.84	76.00 80.00	70.50	71.34	4.20 3.50	1.33	90.00 84.00	76.50	77.83	2.66 2.66	−.14	90.00 84.00	75.00	74.80
Macrocystis pyrifera	1.11	10	0.396	2.10 2.52	.63	3.00 2.90	−1.45	.....	2.52 2.66	.77	6.00 6.00	−1.50	.....	3.22 3.50	.84	10.00 9.00	−1.00	.....	2.94 2.38	−.14	9.00 12.00	−1.50	.....
Do.	5.55	50	1.982	2.80 2.52	.95	.44 .50	−3.93	.....	2.52 2.38	.63	.30 .36	−7.07	.....	7.00 5.60	3.78	8.00 8.00	−2.50	.....	2.94 2.80	.07	14.00 16.00	3.00	.....
Do.	11.11	100	3.962	3.22 2.66	1.26	.20 .13	−4.21	.....	2.38 2.24	.49	.20 .30	−7.25	.....	6.72 5.41	5.04	.35 .60	−10.03	.....	14.01 8.40	8.40	.60 .45	−11.48	.....
Macrocystis pyrifera Dried blood	1.11	10 10	.396	2.66 2.38	.84	7.00 8.00	3.10	39.40	2.32 2.66	.77	12.00 12.00	4.50	52.70	2.66 2.94	.28	14.00 14.00	3.50	37.80	3.64 3.36	.70	20.00 20.00	8.00	87.00
Macrocystis pyrifera Dried blood	5.55	50 10	1.982	3.92 4.90	2.73	1.92 1.92	−2.48	2.50	3.22 3.08	1.33	1.52 1.52	−5.98	None.	4.48 3.22	1.33	15.20 14.00	4.10	54.30	2.52 2.66	.21	22.50 22.50	10.50	107.10
Macrocystis pyrifera Dried blood	11.11	100 10	3.962	4.20 4.62	2.73	.08 .12	−4.30	None.	2.24 2.66	.63	.20 .25	−7.28	None.	13.31 11.07	9.67	.24 .40	−10.18	None.	3.78 2.66	.42	1.00 2.50	−10.25	None.
Macrocystis pyrifera Dried blood	1.11	10 100	.396	17.37 17.65	15.83	50.00 42.00	41.60	57.43	3.22 3.22	1.40	70.00 70.00	62.50	63.90	2.38 2.66	0.00	80.00 80.00	69.50	69.50	2.52 2.94	−.07	76.00 80.00	66.00	65.93
Macrocystis pyrifera Dried blood	5.55	50 100	1.982	44.34 43.81	43.39	2.60 2.80	−1.70	41.63	36.43 35.87	34.33	10.00 12.00	3.50	37.83	4.06 3.92	1.47	70.00 70.00	59.50	60.97	3.36 8.13	2.94	60.00 64.00	50.00	52.91
Macrocystis pyrifera Dried blood	11.11	100 100	3.962	53.10 36.10	42.87	.16 .15	−4.20	38.67	27.34 23.14	23.40	.50 .35	−7.08	16.32	51.70 55.06	50.86	.30 .20	−10.25	40.61	35.16 47.63	38.59	20.00 6.00	1.00	39.59

TABLE IV.—*Series III, part 2. Effect of small and large amounts of dried Nereocystis luetkeana on the rate of ammonification and nitrification of dried blood in clay adobe soil from Berkeley, Cal.*

Material added to 200 gm. of soil.	Quantity of kelp added to 200 gm. soil.	Quantity of nitrogen added.	Quantity of soluble salts in kelp added.	Incubated 2 weeks.						Incubated 4 weeks.					
				Total quantity of nitrogen present in ammonia.	Average quantity of nitrogen gained in ammonia.	Total quantity of nitrogen present in nitrates.	Average quantity of nitrogen gained in nitrates.	Percentage of total nitrogen gained, all calculated to dried blood present.	Percentage of *Nereocystis luetkeana* nitrogen gained.	Total quantity of nitrogen present in ammonia.	Average quantity of nitrogen gained in ammonia.	Total quantity of nitrogen present in nitrates.	Average quantity of nitrogen gained in nitrates.	Percentage of total nitrogen gained, all calculated to dried blood present.	Percentage of *Nereocystis luetkeana* nitrogen gained.
	Gm.	*Mg.*	*Gm.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*			*Mg.*	*Mg.*	*Mg.*	*Mg.*		
Untreated soil	.....	.....	.....	1.54	.....	4.20	.....	.....	.....	1.82	.....	8.00	.....	.....	.....
				1.82		4.60				1.82		7.00			
Dried blood	.....	10	.....	3.94	0.70	12.00	7.60	83.00	.....	1.82	−0.07	14.00	7.00	69.30	.....
				1.82		12.00				1.68		15.00			
Do	.....	100	.....	16.25	14.92	52.00	47.60	62.52	.....	2.52	.84	36.00	70.50	71.34	.....
				16.95		52.00				2.80		80.00			
Nereocystis luetkeana	0.59	10	0.273	1.82	1.40	7.60	3.20	.....	46.00	2.94	1.05	10.00	2.30	.....	33.50
				4.34		7.60				2.80		9.60			
Do	2.94	50	1.366	14.71	13.87	7.00	2.60	.....	32.94	6.02	4.48	12.00	5.00	.....	18.96
				16.39		7.00				6.58		13.00			
Do	5.88	100	2.732	26.90	24.87	3.80	−.80	.....	24.07	28.72	29.77	5.00	−3.00	.....	26.77
				26.20		3.40				34.47		4.00			
Nereocystis luetkeana	.59	10	.....	1.96	.42	12.40	8.00	84.20	1.20	2.80	1.12	16.00	8.50	96.20	26.90
Dried blood	.....	10	.273	2.24		12.40				3.08		16.00			
Nereocystis luetkeana	2.94	50	1.366	13.45	11.21	9.00	5.12	163.10	16.02	7.28	7.42	20.00	11.50	189.20	23.98
Dried blood	.....	10		12.33		10.00				11.21		18.00			
Nereocystis luetkeana	5.88	100	2.732	30.82	29.00	3.60	−.80	282.00	19.90	28.72	30.42	4.00	−3.45	269.7	20.04
Dried blood	.....	10		30.54		3.60				35.73		4.10			
Nereocystis luetkeana	.59	10	.273	12.89	14.01	50.00	44.60	58.61	None	2.52	.84	70.00	61.50	62.34	None.
Dried blood	.....	100		18.49		48.00				2.80		68.00			
Nereocystis luetkeana	2.94	50	1.366	53.80	53.52	20.00	13.60	66.12	3.60	38.53	37.90	30.00	23.00	60.90	None.
Dried blood	.....	100		56.66		14.00				40.91		31.00			
Nereocystis luetkeana	5.88	100	2.732	83.36	79.37	4.00	−.40	78.97	16.45	91.77	86.93	6.00	2.00	88.93	17.59
Dried blood	.....	100		78.74		4.00				85.74		5.00			

The data obtained where dried blood alone was added do not call for any special comment. The production of ammonia and nitrates, with some minor variations, proceeds through each period. When Macrocystis and blood are present, the conditions are very different. The blood is a very available material; the Macrocystis is slowly available. It is therefore reasonable to suppose that the first gains of ammonia and nitrates will be due to the blood. Later, the Macrocystis may also furnish a portion of the nitrogen gained. If at all times the percentage of total gain is calculated as if it were due to the nitrogen of the blood, it can readily be seen whether the total amount gained is greater or less than that where the blood alone was present. This has accordingly been done. If the kelp also furnishes a large part of the gain, the result will evidently exceed 100 per cent and will clearly indicate this fact. It is not claimed that this procedure is strictly accurate, but it will demonstrate whether as much nitrogen is gained with blood and kelp as with blood alone.

With two weeks' incubation we find that this is not so. All quantities of Macrocystis give a reduction in the total gain. Ten mg. of nitrogen in kelp added to one hundred in blood show the smallest inhibitive effect. At four weeks the results are practically the same. Eleven weeks' incubation shows a distinct reduction of the inhibition; especially is this so in regard to nitrification. At 15 weeks it is found that with 10 mg. of blood and with both 10 and 50 mg. of kelp inhibition has ceased and there has been a gain from the kelp added. With the 100-mg. tumblers of blood and Macrocystis nitrification is observed to have commenced. The general evidence would appear to be that at first there undoubtedly is an inhibitive effect from the Macrocystis. This inhibition is gradually reduced as time goes on.

The results from the two sets of tumblers with dried and ground Nereocystis furnish a very striking contrast to those from the Macrocystis. With the kelp alone there is a very ready and uniform conversion to both ammonia and nitrates. It is only with the 100-mg. portions that a very marked inhibition of nitrification has occurred.

The total gain of ammonia and nitrates has again been calculated as if it were all due to blood. The resulting percentages in a number of cases show a greater gain than occurred where blood alone was added to the soil. The difference is naturally assumed to be due to the Nereocystis present. This difference between the blood alone and blood plus Nereocystis has been calculated as the percentage of Nereocystis nitrogen gained. The data throughout show that the Nereocystis is a very available material. This corroborates the results obtained in Series I, which showed that it readily ammonified. We have now proved in addition that it also nitrifies rapidly.

SERIES IV.—COMPARATIVE AMMONIFICATION AND NITRIFICATION OF MACROCYSTIS, FRESH, AIR-DRY, HIGHLY HEATED, AND PARTIALLY DRY

The results of Series III showed that the air-dry Macrocystis with which we are principally concerned is a slowly available material. Series I and II indicated that the kelp which had been dried to a constant weight for a number of hours was less available. It therefore appeared desirable to find what degrees of availability there would be between the thoroughly dry kelp and the fresh material. It was quite possible that this might throw some light on the most desirable method of handling the kelp commercially. One patent has been taken out for a process by which the kelp is heated to from 250° to 270° C. and thoroughly "parched." Many advantages for this process are claimed.

A series of experiments were therefore conducted at Pacific Grove. In these *Macrocystis pyrifera* was air-dried completely, so that it contained only 8.68 per cent of moisture. Duplicate portions were dried so that they contained 28.63, 37.15, and 55.32 per cent of moisture. Fresh kelp was also gathered from the same bed. On returning to Berkeley, two duplicate portions of the air-dry kelp of 500 gm. each were heated to from 250° to 270° C. till thoroughly parched. During this process 36 per cent of the organic matter present was lost, and in this organic matter driven off 31 per cent of the nitrogen originally present in the kelp was also lost.

The materials were all used in an ammonification and nitrification study which was conducted for three weeks. The soil used was clay adobe, as in Series III. Two portions of each sample of kelp were added to triplicate sets of tumblers. One weight of kelp used contained just 0.27 gm. of soluble salts, while the other contained just twice as much, 0.54 gm. (See Table V.)

It will be observed that the fresh kelp as before gave a very satisfactory conversion to ammonia and nitrates. The air-dry Macrocystis with the smaller quantity added gave a negative result. With double the amount of kelp there was a small but consistent gain. The parched kelp gave no conversion whatsoever. The partially dry kelp was all fairly available. That which contained 28 per cent of moisture was, in fact, more readily changed than the fresh sample. The evident conclusion is that Macrocystis will be more readily decomposed if it can be handled without drying completely. It should be noted that the samples with 28 and 37 per cent of moisture could be kept without danger of decay or mold. That with 55 per cent was too moist to be safely stored.

TABLE V—*(Series IV). Ammonification and nitrification of Macrocystis pyrifera, fresh, air-dry, highly heated, and partially dry. Incubated for three weeks*

Material added to 200 gm. of soil.	Percentage of moisture in kelp.	Quantity of kelp added.	Quantity of nitrogen added.	Quantity of soluble salts in kelp added.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Quantity of nitrogen gained in ammonia and nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.
		Gm.	*Mg.*	*Gm.*	*Mg.*	*Mg.*	*Mg.*	
Macrocystis pyrifera, fresh	87.00	7.00	19.45	0.27	0.23	5.14	5.37	20.67
					.23	3.94	4.17	
					−.05	2.59	2.54	
Do	87.00	14.00	38.90	.54	.37	9.27	9.64	27.97
					.51	11.27	11.78	
					−.05	11.27	11.22	
Macrocystis pyrifera, air-dry	8.68	.72	10.35	.27	.65	−1.56	−.91	None.
					.65	−1.56	−.91	
					.51	−1.56	−1.05	
Do	8.68	1.44	20.70	.54	.65	.94	1.59	8.21
					.79	.94	1.73	
					1.35	.44	1.79	
Macrocystis pyrifera, heated to 250° C.	1.44	.50	8.43	.27	−.19	−1.56	−1.75	None.
					.65	−1.56	−.91	
					.23	−2.06	−1.83	
Do	1.44	1.00	16.86	.54	.09	−1.56	−1.47	None.
					.51	−2.06	−1.55	
					.09	−2.06	−1.97	
Macrocystis pyrifera	28.63	.99	12.32	.27	.23	3.44	3.67	28.98
					−.05	3.44	3.39	
					.23	3.44	3.67	
Do	28.63	1.98	24.64	.54	2.19	5.94	8.13	29.02
					1.07	5.94	7.01	
					.37	5.94	6.31	
Do	37.15	1.09	10.73	.27	.51	.94	1.45	15.58
					.09	.94	1.03	
					.65	.94	1.59	
Do	37.15	2.19	21.46	.54	.37	4.94	5.31	27.59
					1.35	5.94	7.29	
					.23	4.94	5.17	
Do	55.32	1.34	10.20	.27	.09	1.94	2.03	23.04
					.79	1.94	2.73	
					.37	1.94	2.31	
Do	55.32	2.68	20.40	.54	1.07	3.44	4.51	24.12
					.37	4.94	5.31	
					1.21	3.44	4.65	

GENERAL DISCUSSION OF RESULTS

The preceding studies on the availability of the major kelps of the Pacific coast demonstrate a number of important facts, which are consistently shown by all the series of experiments. *Nereocystis luetkeana*, which is not commercially important, proved to be the most available. It is not a highly nitrogenous substance, like dried blood or cottonseed meal. We should not, therefore, expect it to be so readily decomposed as these materials. The rate of ammonification and nitrification which it has shown in Series I and III is therefore considered to be very satisfactory.

Pelagophycus porra ranks next to the Nereocystis in availability, while *Macrocystis pyrifera*, the commercial variety, is the least available of all. The studies carried through with this material prove that when very completely oven-dried this kelp is changed in the soil with extreme slowness. When sun-dried so that it can be readily ground, it requires about 11 weeks to ammonify and to begin to nitrify appreciably. In Series IV a slight gain is shown by one quantity of air-dry Macrocystis at the end of three weeks. This kelp was dried for the shortest possible time in the sun and the drying was then completed in the incubator room at 28° C. The other samples in Series IV, which contained greater amounts of moisture, were all more available. It would therefore appear that Macrocystis should be dried and ground at as low a temperature as possible. Commercially, artificial heat will probably be necessary for drying. This drying will have to continue till the kelp is crisp and practically water-free, but should not be carried on for a longer time than may be necessary to have it attain this condition.

The addition of moderate quantities of Nereocystis to the soil has not caused any great inhibition to either ammonification or nitrification. With Macrocystis, however, very appreciable inhibition is at first shown. As time goes on, this is gradually less evident. With the smaller amounts of kelp used in field fertilization, the inhibition would undoubtedly be less and would probably be sooner counteracted.

CONCLUSIONS

(1) In preparing dried and ground kelp as a fertilizer the availability or readiness with which the nitrogen in it is changed to ammonia and nitrates in fresh field soil was found to vary with different species and with the manner of preparation.

(2) The nitrogen of *Nereocystis luetkeana* is relatively very available, while that of *Pelagophycus porra* is less readily changed. These two varieties are of minor commercial importance.

(3) *Macrocystis pyrifera*, the commercial variety, is very slowly changed in the soil.

(4) The availability of the nitrogen of *M. pyrifera* is greatest when the kelp is added in a fresh or only partially dried condition.

(5) The availability of its nitrogen decreases materially when Macrocystis is fully dried.

(6) Removing a large portion of the salts from either fresh or dry Macrocystis by leaching does not cause it to decompose more readily.

(7) Macrocystis must be dried till crisp in order to grind readily. This drying should not be continued longer than necessary, and the kelp should not be scorched or overheated.

(8) The addition of moderate quantities of Nereocystis to a sample of fresh soil in the laboratory did not cause any great interference with

either ammonification or nitrification of readily available organic matter, such as dried blood.

(9) Similar experiments with Macrocystis showed at first a decrease in the rate of transformation, especially in nitrification. This decrease did not continue and as time passed the ammonification and nitrification became practically normal.

(10) When using kelp in field practice, it is probable there would be no interference with either ammonification or nitrification from either the kelp or the salts present in it.

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ORGANIC CONSTITUENTS OF PACIFIC COAST KELPS

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INTRODUCTION AND PLAN OF WORK

The giant kelps of the Pacific coast have been regarded during recent years as commercially profitable sources of potash and iodin. The high content of these constituents in the kelp was first given prominence by Balch (1),[1] and later the Bureau of Soils of the United States Department of Agriculture (4) made further studies and mapped out many of the beds. These investigations were followed by a widespread interest in kelps and it was the prevailing idea that these plants would furnish the raw material for industries of considerable magnitude. It seemed, however, that such predictions required further verification through more extended chemical studies than were available, since in many directions exact information was entirely lacking. Accordingly the Chemical Laboratory of the California Experiment Station during the past year has carried on a general investigation of the subject the principal results of which are discussed in publications of this Station by Burd (3) and Stewart (32).

While the potash and iodin values have, as a matter of course, received first attention in all discussions of a kelp industry, it has been apparent that any commercially valuable by-products of an organic nature would greatly enhance the possibilities of utilizing kelp with a margin of profit. Practically no studies of the organic constituents of the California kelps have been made prior to the writer's, and it is with this aspect of the investigations that the present paper deals.[2] It is not the intention to regard the experiments herein described as forming in any sense a complete and final study of the numerous questions involved. The composition and chemical physiology of these marine algæ are in many respects unique. There are obviously infinite possibilities for studies of great scientific interest along these lines. If opportunity is afforded, more detailed experiments in regard to the nature of certain interesting constituents of the kelp will be conducted in this laboratory. It is, however, deemed advisable to present such results and conclusions as are now at hand, owing to the considerable interest recently manifested in this subject.

The conceivable uses for the organic matter of kelp which have most frequently been mentioned include destructive distillation, feeding of

[1] Reference is made by number to "Literature cited," p. 56–58.

[2] Acknowledgment is made to Mr. W. H. Dore, of the Chemical Laboratory of the California Experiment Station, for much assistance in the analytical work and suggestions as to methods.

animals, production of sizes, glues, or varnishes, clarification of wines, and paper making. In these studies on the kelp the writer has endeavored to obtain analytical and other data essential to a more accurate judgment in regard to the above possibilities, at the same time having in mind certain factors of purely theoretical interest. For greater convenience of discussion the work is described under the following subheads: General study of important chemical groups; Forms of nitrogen; Carbohydrates; Cellulose; Hydrolysis; Sulphur content; Forms of iodin; Economic considerations, including the results of destructive distillation.

Several varieties of seaweeds have been utilized commercially in Japan for over two centuries. They have served as a basis for the preparation of "Kombu" and other foodstuffs, for glue manufacture, and for the manufacture of "Kanten," in extensive use under the name "agar-agar" (22). The commercially important seaweeds of Japan are, however, of different species and undoubtedly have chemical properties distinct from those of the giant kelps (*Macrocystis* spp. and *Nereocystis* spp.) of the Pacific coast.

The earliest chemical studies of seaweed were made by Stanford (24–31) on European species about 40 years ago. He described a product obtained from kelp, which he called "algin" or "alginic acid," and for which he claimed remarkable properties. Many uses were suggested for this substance: A sizing material for fabrics, a glue, a food, a water-softening reagent, and various other applications. A theoretical formula was deduced for this so-called "algin," and its elementary composition was stated, but Stanford presented no systematic or detailed chemical studies in support of his conclusions. Furthermore the chemical methods available at that period were very imperfect.

Since the various papers of Stanford (24–31) were published, only occasional articles of very limited scope have appeared on the organic chemistry of the important marine algæ. For the giant kelp of the Pacific coast no systematic analyses or chemical studies of the organic matter have been made, although there are many assertions regarding the presence of valuable substances, such as rubber, useful varnishes, or commercially profitable distillation products. These points will be discussed under their appropriate headings.

GENERAL STUDY OF IMPORTANT CHEMICAL GROUPS

The limitations in value of the so-called proximate analyses by the "official" methods are recognized. Such data at present seem, nevertheless, to be essential for purposes of general classification and comparison. In the accompanying tables an extended series of such analyses is presented. The following species of kelp were investigated: *Macrocystis pyrifera, Nereocystis luetkeana, Pelagophycus porra, Egregia laevigata, Egregia menziesii, Laminaria andersonii, Iridaea* sp. Analyses were made

on the analytical samples collected by Mr. Guy R. Stewart, of this laboratory. Every precaution was taken to insure fair and representative samples, as described in the preceding paper (32). Tabulations are made on the water-free basis and also on the original material. In the consideration of possible utilization of kelp products proper emphasis should be given to the high percentage of water in the plant.

A qualitative examination of the varieties of kelp investigated indicates the presence of very complex compounds in a highly colloidal state. Starch and soluble reducing sugars are absent from these plants. In all cases strong tests for furfural (pentosan test) are given, and some samples gave the galactan test by oxidation with nitric acid. The major portion of the total organic matter present is insoluble in water and in alcohol. These relations are given in Table I.

TABLE 1.—*Organic matter in kelp*

No. of sample.	Species of kelp and part used.	Percentage of total organic matter	Percentage of plant.		Percentage of total organic matter.	
			Water-soluble organic matter.	Alcohol-soluble organic matter.	Water-soluble organic matter.	Alcohol-soluble organic matter.
42	*Egregia* spp	67. 87	10. 08	6. 84	14. 9	10. 1
43	*Laminaria andersonii*	73. 80	23. 32	8. 56	31. 6	11. 6
44	*Iridaea* sp	69. 12	25. 80	8. 16	37. 3	11. 8
45. 1	*Nereocystis luetkeana*, leaves	47. 27	18. 88	12. 72	39. 9	26. 9
45. 2	*Nereocystis luetkeana*, stems	39. 02	13. 24	6. 68	33. 9	17. 1
46. 1	*Macrocystis pyrifera*, leaves	54. 30	11. 56	8. 36	21. 3	15. 4
46. 2	*Macrocystis pyrifera*, stems	51. 22	25. 40	13. 12	49. 6	25. 6
47. 1	*Pelagophycus porra*, leaves	58. 60	23. 32	18. 04	39. 8	30. 8
47. 2	*Pelagophycus porra*, stems	37. 35	14. 40	9. 28	38. 5	24. 9

It may be noted in this connection that certain varieties of marine algæ, investigated in other parts of the world, have shown different chemical characteristics. Levulose, mannit, starch, and easily hydrolyzable polysaccharids have been reported (2, 18).

Table II gives the general composition of the various portions of the kelp plants for the different species investigated. The extent of the variations between individual plants is indicated in Table III. The total organic matter varies from one-third to three-fourths of the dry weight. Using the conventional factor of 6.25 for nitrogen, we have as high as 17 per cent of protein if all the nitrogen existed in that form. (The forms of nitrogen will be discussed later.) The plants of Macrocystis obtained at Pacific Grove are distinctly higher in this constituent, as compared with the samples of Macrocystis from the vicinity of San Diego. In none of the species is the percentage of ether extract important, the average exceeding 1 per cent in only the one case of Nereocystis

leaves. A considerable portion of the ether extract is, of course, coloring matter and not true fat. The percentages of crude fiber are not high, from 6 to 10 per cent being fairly constant for all samples. The same statement applies to the pentosans. *Iridaea* sp. (a rockweed) is exceptional in this respect, having less than 1 per cent as pentosans. Numerous attempts were made to estimate by the mucic-acid method the proportion of galactans present, but in most cases entirely untrustworthy results were obtained. The method was found to be inherently unreliable and especially unadaptable to materials of this type. Approximate results were obtained for the Pelagophycus and the Iridaea, the former yielding mucic acid equivalent to 3 per cent of galactan, the latter 10 per cent.

TABLE II.—*Average composition of the organic matter of Pacific coast kelp, calculated on whole plant*

Material used.	Number of samples analyzed.	Percentage composition calculated to fresh material.									Percentage composition calculated to a water-free basis.							
		Moisture.	Total salts.	Total organic matter.	Nitrogen, 6.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate.	Sodium carbonate soluble acid precipitate (algin).	Total salts.	Total organic matter.	Nitrogen, 6.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate.	Sodium carbonate soluble acid precipitate (algin).
Macrocystis pyrifera, harvestable portion, San Diego	8	80.3	5.24	8.45	1.02	0.046	0.98	1.06	0.91	2.56	38.2	61.7	7.4	0.34	7.2	7.7	6.6	18.7
Macrocystis pyrifera, harvestable portion, Pacific Grove	4	87.7	5.28	7.00	1.65	.049	.93	.77	1.07	1.77	42.9	56.9	13.4	.40	7.6	6.3	8.7	14.4
Macrocystis pyrifera, nonharvestable portion, San Diego	8	87.7	5.16	7.11	1.33	.054	.89	.98	1.08	2.15	42.0	57.8	10.8	.44	7.2	8.0	8.8	17.5
Nereocystis luetkeana, entire plant, Pacific Grove	4	91.7	4.52	3.74	.90	.088	.42	.53	.71	1.17	54.5	45.1	10.8	1.06	5.1	6.4	8.6	14.1
Pelagophycus porra, entire plant, San Diego	2	89.7	5.43	4.91	.77	.028	.64	.87	.69	1.66	52.7	47.7	7.5	.27	6.2	8.4	5.8	16.1
Egregia laevigata, entire plant, San Diego	2	83.7	5.78	10.52	1.89	.144	1.47	1.60	1.48	3.04	35.5	64.5	11.6	.88	9.0	9.8	9.1	18.7
Egregia menziesii, entire plant, Pacific Grove	3	83.6	5.48	10.86	2.82	.110	1.43	2.48	.90	3.14	33.4	66.3	17.2	.67	8.7	9.0	5.5	19.1
Laminaria andersonii, entire plant	1	78.5	5.70	15.80	3.22	.140	2.24	2.15	.37	4.90	26.5	73.5	15.0	.65	10.4	10.0	1.7	22.8
Iridaea sp., entire plant	1	80.1	6.25	13.70	3.38	.087	2.09	.18	.26	.20	31.4	68.8	17.0	.44	10.5	.9	1.3	1.0

TABLE III.—*Maximum, minimum, and average composition of organic matter of leaves and stems of Pacific coast kelp*

Material used.	Number of samples.	Percentage composition calculated to fresh material.									Percentage composition calculated to a water-free basis.							
		Moisture.	Total salts.	Total organic matter.	Nitrogen, 0.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate	Sodium carbonate soluble acid precipitate (algin).	Total salts.	Total organic matter.	Nitrogen, 0.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate	Sodium carbonate soluble acid precipitate (algin).
Macrocystis pyrifera, maximum composition, harvestable leaves, San Diego and La Jolla	8	88.9	6.26	11.99	1.75	0.065	1.37	1.38	1.06	3.72	40.9	71.4	10.8	0.43	9.0	9.1	7.0	24.5
Macrocystis pyrifera, minimum composition, harvestable leaves, San Diego and La Jolla	8	84.2	4.44	7.22	.87	.035	.72	1.05	.55	2.97	30.4	60.2	5.2	.21	6.0	6.5	3.7	16.4
Macrocystis pyrifera, average composition, harvestable leaves, San Diego and La Jolla	8	85.3	5.22	9.90	1.27	.045	1.04	1.13	.73	2.71	35.5	64.5	8.6	.31	7.1	7.7	5.0	18.4
Macrocystis pyrifera, average composition, harvestable leaves, Pacific Grove and vicinity	4	86.8	5.33	7.87	2.19	.046	1.07	.84	.90	1.80	40.4	59.6	16.6	.35	8.1	6.4	6.8	13.6
Macrocystis pyrifera, maximum composition, harvestable stems, San Diego and La Jolla	8	88.2	5.99	7.73	.92	.061	1.07	1.05	1.55	2.66	48.4	61.8	8.1	.55	8.1	8.7	12.4	22.0
Macrocystis pyrifera, minimum composition, harvestable stems, San Diego and La Jolla	8	85.6	4.78	5.63	.84	.033	.71	.91	.53	1.92	42.3	49.8	12.6	.30	7.2	8.2	4.4	17.1
Macrocystis pyrifera, average composition, harvestable stems, San Diego and La Jolla	8	88.2	5.29	6.48	.55	.029	.85	.94	1.24	2.28	44.8	54.9	4.7	.42	7.2	8.0	10.5	19.3
Macrocystis pyrifera, average composition, harvestable stems, Pacific Grove and vicinity	4	89.3	5.20	5.52	.74	.054	.69	.65	1.35	1.72	48.6	51.6	6.9	.51	6.5	6.1	12.6	16.1
Macrocystis pyrifera, average composition, nonharvestable leaves, San Diego and La Jolla	6	86.9	5.54	7.56	1.95	.056	.94	.94	.95	2.11	42.3	57.7	14.9	.43	7.2	7.2	7.3	16.1
Macrocystis pyrifera, average composition, nonharvestable leaves, Pacific Grove and vicinity	2	86.5	5.51	8.03	2.14	.063	.74	.79	.53	1.76	40.8	59.5	15.9	.47	5.5	5.9	3.9	13.0
Macrocystis pyrifera, average composition, nonharvestable stems, San Diego and La Jolla	6	88.4	4.83	6.73	.80	.051	.86	1.01	1.18	2.18	41.6	58.0	6.9	.44	7.4	8.7	10.2	18.8
Macrocystis pyrifera, average composition, nonharvestable stems, Pacific Grove and vicinity	2	88.6	5.08	6.27	.75	.055	.78	.71	.96	1.84	44.6	55.0	6.6	.48	6.6	6.2	8.4	16.1

Nereocystis luetkeana, average composition, leaves, Pacific Grove and vicinity	1	91.3	4.49	4.27	1.09	1.16	.41	.55	.76	1.45	39.6	49.1	1.25	1.45	41.7	6.3	8.7	14.4
Nereocystis luetkeana, average composition, stipes, Pacific Grove and vicinity	1	90.1	4.20	2.88	.57	.025	.44	.49	.64	1.63	61.8	65.9	2.5	.81	3.8	10.5	8.4	13.6
Pelagophycus porra, average composition, leaves, San Diego and vicinity	1	86.0	5.70	6.25	.99	.034	.73	.99	.55	1.61	47.5	55.1	8.2	.25	6.1	8.2	4.6	10.1
Pelagophycus porra, average composition, stipes, San Diego and vicinity	2	91.8	5.06	3.14	.49	.023	.54	.72	.67	1.39	61.7	38.3	0.0	.28	0.8	8.8	8.2	15.9
Eurcgia laciniata, average composition, complete, San Diego and vicinity	2	83.7	5.78	10.52	1.59	.144	1.47	1.66	1.48	3.04	35.5	64.5	11.6	.88	9.0	6.8	9.1	18.7
Eurcgia laciniata, average composition, complete, Pacific Grove and vicinity	3	83.6	5.48	10.68	.102	.110	1.43	1.48	.90	3.14	55.4	66.1	12.2	.69	8.7	9.0	5.5	19.1
Laminaria andersonii, composition, complete	1	78.5	5.79	15.96	3.45	.140	2.84	5.18	.47	4.90	76.4	23.6	15.0	.19	10.4	10.0	1.7	22.8
Iridaea spp., composition, complete	1	76.1	6.25	13.79	3.35	.065	2.69	.18	.20	3.14	76.4	23.6	17.6	.42	10.8	.9	1.3	2.6

FORMS OF NITROGEN PRESENT

In order to estimate accurately the value of nitrogenous substances, more than a mere statement of the percentage of nitrogen is necessary. All compounds of nitrogen are not of equal worth, especially in nutrition. Experiments were therefore undertaken to determine the general forms of nitrogen present in the kelp. Water-soluble nitrogen, alcohol-soluble nitrogen, and nonprotein nitrogen by Stutzer's reagent, also by phosphotungstic acid, were estimated. Extractions were made on dried and finely ground samples. An important part of the nitrogen (from one-fifth to one-third) is soluble in cold water. (See Table IV.) About the same proportion is classed as nonprotein nitrogen, using the methods just mentioned. Other experiments, in which fresh kelp was leached, showed similar relations for soluble and insoluble nitrogen. The water extracts from a number of samples of ground kelp were analyzed for acid amid and ammonia nitrogen, using the method of Abderhalden. Protein nitrogen was precipitated by means of phosphotungstic acid, the filtrate boiled in an 8 per cent solution of hydrochloric acid and distilled in the presence of an excess of magnesium oxid. In all cases the amount of nitrogen so estimated was insignificant.

TABLE IV.—*Forms of nitrogen in kelp*

No.	Material used.	Percentage of dried and ground sample.					Percentage distribution of nitrogen.			
		Total nitrogen.	Nonprotein nitrogen. By Stutzer's reagent.	Nonprotein nitrogen. By phosphotungstic acid.	Water-soluble nitrogen.	Alcohol-soluble nitrogen.	Nonprotein nitrogen. By Stutzer's reagent.	Nonprotein nitrogen. By phosphotungstic acid.	Water-soluble nitrogen.	Alcohol-soluble nitrogen.
1. 1	*Macrocystis pyrifera* No. 1, harvestable leaves	1.69	0.55	0.42	0.58	0.18	32.5	24.9	34.3	10.7
1. 2	*Macrocystis pyrifera* No. 1, harvestable stems	.98	.30	.32	.38	.12	30.6	32.7	38.8	12.2
1. 3	*Macrocystis pyrifera* No. 1, nonharvestable leaves	2.19	.89	.70	.99	.32	37.1	29.2	41.2	13.3
9. 1	*Macrocystis pyrifera* No. 9, harvestable leaves	1.01	.19	.18	.20	.06	18.8	17.8	19.8	6.0
9. 3	*Macrocystis pyrifera* No. 9, nonharvestable leaves	1.62	.32	.25	.41	.10	19.8	15.4	25.3	6.2
15. 1	*Pelagophycus porra*, No. 4, leaves	1.24	.22	.18	.23	.18	17.7	14.5	18.5	14.5
15. 2	*Pelagophycus porra* No. 4, harvestable stem	.98	.15	.16	.23	.07	15.3	16.3	23.5	7.1
27. 1	*Nereocystis luetkeana* No. 1, leaves	1.86	.50	.37	.52	.35	26.9	19.9	28.0	18.8
28. 1	*Nereocystis luetkeana* No. 2, leaves	1.90	.55	.48	.58	.43	29.0	25.3	30.5	22.6
29. 1	*Nereocystis luetkeana* No. 3, leaves	2.20	.65	.57	.73	.47	29.5	25.9	33.2	21.3
29. 2	*Nereocystis luetkeana* No. 3, stem	1.05	.24	.21	.28	.15	22.9	20.0	26.7	14.3
30. 1	*Nereocystis luetkeana* No. 4, leaves	1.97	.38	.45	.55	.32	24.4	22.8	27.9	16.2
42	*Egregia menziesii* No. 3, complete strands	2.75	.43	.37	.48	.24	15.6	13.5	17.5	8.7
43	*Lammaria andersonii*, entire plants	2.38	.48	.41	.64	.32	20.2	17.2	27.3	13.4
44	*Irideae* sp., entire plants	2.67	.52	.53	.58	.36	19.5	19.8	21.7	13.5

CARBOHYDRATES IN KELP

Carbohydrates or analogous bodies make up the principal portion of the organic matter. The carbohydrates of these algæ are complex colloidal substances which would ordinarily be classified among the vegetable gums, or pectins. Very little information is obtainable for these groups, and there are no satisfactory specialized chemical methods available for their study. In general, complex mucilaginous polysaccharids are characteristic of marine algæ (8, T. 1, p. 68), replacing the starch, cellulose, and simple sugars of most land plants. Even where starch and simple carbohydrates have been reported to be present in algæ, the amounts are relatively small. The physical properties form the most important consideration in the utilization of the carbohydrates of the algæ. From some products valuable jellies may be prepared, for example, agar-agar. The California kelps studied in this laboratory do not have this property of jellification to any valuable degree.

ALGIN

The fraction to which the name "algin" has been given is quantitatively and in point of interest the most important of the carbohydrate constituents of kelp. Briefly described, algin may be separated from the seaweed in the following manner: The material is digested cold for 24 hours with a dilute solution of sodium carbonate or other alkali. A very thick, sirupy mixture results, which is filtered with suction. The filtrate is treated with a slight excess of sulphuric or hydrochloric acid. Immediately a heavy yellow precipitate is formed and floats in the watery liquid. In its water-holding power this body may well be compared with a sponge. On exposure to the air the color of the moist precipitate rapidly deepens to a dark brown, and on drying it shrinks to a dark-colored hard substance.

Stanford, in the investigations already mentioned (24–31) in this paper, concluded that algin prepared in this manner was a definite chemical body. He assumed that nitrogen was an essential constituent and even advanced a definite formula: $C_{70}H_{76}O_{22}\begin{matrix}NH_2\\NH_2\end{matrix}$. Such a formula is without justification, since the elementary analysis was made on a highly contaminated sample. Stanford further described a series of salts of alginic acid with the heavy metals, and these, as well as the original algin, he believed to be of considerable commercial importance. Smith (21), in summarizing this and other work, compares algin with cottage cheese and quotes the following analysis made by Stanford: C, 44.39 per cent; H, 5.47 per cent; N, 3.77 per cent; O, 46.37 per cent.

More recently Kylin (15) has described an algin which he prepared from *Laminaria digitata* and from several related species. Many of the

characteristics correspond to those described by Stanford, although Kylin made his original extraction with water rather than alkali.

In this laboratory experiments were inaugurated to determine to what extent a similar substance might be present in the Pacific coast kelps, and in addition its general chemical characteristics. It was found that the maximum yield of algin was obtainable by digestion in the cold and with a dilute sodium-carbonate solution (2 per cent). The use of stronger alkalies or the application of heat is unfavorable, probably because of a tendency of the body to decompose under such conditions. The following procedure was adopted for the estimation of the algin fraction:

Two gm. of kelp were digested in the cold for 24 hours with a 2 per cent sodium-carbonate solution. The residue was filtered off and washed with cold water. Twenty c. c. of dilute hydrochloric acid were added to the filtrate, and the precipitate was allowed to stand for 24 hours. It was then filtered on a linen cloth, washed, dried, weighed, ignited, and the weight of the ash subtracted.

The percentages obtained by this method varied from 13 to 24, calculated on the dry kelp. (See Table III.) *Iridaea* spp. again form an exception, having only 1 per cent of this complex. The general properties of the algin thus obtained are as follows: Solubility in sodium carbonate, ammonia, and other alkalies, with formation of viscous solutions exceedingly difficult of filtration; insolubility in water, in strong acids, in alcohol, also in ether, benzine, turpentine, etc.; resistance to solution after complete drying; precipitation by copper and other heavy metals. Long standing in even a weak alkaline solution causes a decomposition or fermentation to take place, so that precipitation with acid is no longer possible.

A sample of algin was subjected to further purification by three repre cipitations with hydrochloric acid and two by alcohol from the slightly alkaline solution. The product was finally bleached with sulphurous acid, and was thoroughly washed and dried. The following analytical results were obtained:

	Per cent.
Nitrogen	0.3
Ash	2.2
Furfural calculated to pentosan	38.6
Insoluble after treatment with concentrated nitric acid (cellulose derivative)	24.5

Some of the moist precipitate was boiled for several hours with a 2 per cent solution of sulphuric acid. The solution gave a good reduction of Fehling's solution. Drying caused the substance to become very much more resistant to hydrolysis. Treatment with nitric acid did not give the mucic-acid test for galactan. We may conclude from these observations that algin is a very complex resistant compound (or mixture of com-

pounds) of the pentosan type, with cellulose possibly making up a part of the complex. Algin has weakly acid properties, forming soluble compounds[a] with the alkali metals. Thus, the sodium alginate precipitated by alcohol is easily soluble in water, while the alginic acid is almost insoluble in water. To precipitate out the alginic acid requires apparently a definite concentration of the hydrogen ion. No precipitation occurs with the weakly dissociated organic acids unless present in excess. From an acetic-acid solution of algin, gelatinous precipitates may be obtained with a large number of metallic salts. Mr. L. L. Lieb, of this laboratory, has prepared a series of such compounds, as described in Table V.

TABLE V.—*Metallic alginates*

SOLUBLE ALGINATES (PRECIPITATED BY ALCOHOL)[a]

Metal.	Metallic salt used.	Color of fresh precipitate.	General properties of precipitate.
Li	Li (acetate)	Silver white	Gelatinous, transparent.
Na	NaOH	White	"Stringy," brittle when dry.
Mg	Mg (acetate)	do	Transparent, gelatinous.
NH_4	NH_4OH	Light yellow	Light, gelatinous.
K	KOH	Transparent	Light, fluffy.

INSOLUBLE ALGINATES (PRECIPITATED FROM ACETIC ACID SOLUTION)

Al	$AlCl_3$	White	Gelatinous, brittle when dry, brown color.
Ca	$CaCl_2$	do	Gelatinous, glossy when dry.
Cr	$Cr(NO_3)_3$	Light blue	Heavy, nongelatinous.
Mn	$Mn(C_2H_3O_2)_2$	Light red	Gelatinous, good gloss to paper when dry.
Fe″	$FeSO_4.7H_2O$	Light brown	Gelatinous, brittle when dry.
Fe‴	$FeCl_3$	Brown	Gelatinous.
Co	$Co(NO_3)_2$	Reddish	Gelatinous, good gloss to paper when dry.
Ni	$NiCl_2$	Light green	Gelatinous.
Cu	$CuSO_4$	do	Do.
Zn	$ZnSO_4$	Colorless	Gelatinous, silvery gloss to paper when dry.
Sr	$Sr(NO_3)_2$	Light brown	Heavy gelatinous, transparent when dry.
Ag	$AgNO_3$	White	Gelatinous, becomes dark red when dry.
Cd	$Cd(NO_3)_2$	Colorless	Gelatinous, becomes horny.
Sn″	$SnCl_2H_2O$	White	Thick, gelatinous.
Sn‴	$SnCl_4$	do	Do.
Ba	$BaCl_2$	do	Gelatinous, good gloss to paper when dry.
Pt[b]	$PtCl_4$	Light brown	Gelatinous.
Au[b]	$AuCl_3$	Red	Do.
Hg′	$Hg_2(NO_3)_2$	White	Dense, white, gelatinous.
Pb	$Pb(C_2H_3O_2)_2$	Colorless	Gelatinous, like isinglass when dry.
Bi	$Bi(NO_3)_2$	White	Gelatinous.
U	$U(SO_4)_2.4H_2O$	Yellow	Thick, gelatinous.

[a] All these soluble alginates give more or less gloss to paper. [b] Precipitated from alcohol solution

Stanford (30) prepared a considerable number of compounds similar to those described here, although his data presented some discrepancies. An inspection of the table will indicate certain theoretical possibilities for the use of alginates as sizes or mordants, but the practical difficulties of preparation would probably prevent any commercial application of such products in competition with the various low-priced materials now used for these purposes.

CARBOHYDRATES PRECIPITABLE BY ALCOHOL

The alcohol-precipitable matter was prepared by treating the dried and ground kelp with a weakly acid solution, and adding sufficient strong alcohol to the filtrate to cause a complete precipitation. The flocculent precipitate was filtered by suction and washed with alcohol; the weight of the ash was subtracted from the total dry weight. The product very readily absorbed water from the atmosphere, soon becoming mucilaginous. The dried substance contained 1.2 per cent of nitrogen and yielded furfural, corresponding to 13.2 per cent of pentosan. No color test was given with iodin and no reduction with Fehling's solution. The moist precipitate was boiled several hours with a 2 per cent solution of sulphuric acid when the solution produced considerable reduction of the alkaline copper solution. Upon drying, the precipitate became very resistant to solution and to hydrolysis. The percentages in the plant of the carbohydrate bodies precipitated by alcohol are much less than for algin. (See Table III.) In the plants of *Macrocystis pyrifera* the stems show uniformly higher percentages than the leaves.

CELLULOSE

A composite sample of fiber, obtained as in the crude fiber method, was treated by the method of Cross and Bevan (5, p. 94–95), by chlorination and boiling with alkaline sodium sulphite. Final decolorization was effected with potassium permanganate. Pure cellulose was thus obtained and was found to make up approximately one-half of the crude fiber, or calculated on the whole dry plant, 3 to 4 per cent.

HYDROLYSIS OF KELP

Acid hydrolysis of the dried kelp yielded copper-reducing substances only with great difficulty. Ten hours' boiling with a 2 per cent sulphuric-acid solution gave the following amounts of reduced copper calculated as dextrose:

	Per cent.		Per cent.
Macrocystis pyrifera, leaves	6.4	*Nereocystis luetkeana*, stems	5.7
Macrocystis pyrifera, stems	7.0	*Egregia menziesii*	9.9
Pelagophycus porra, leaves	9.0	*Laminaria andersonii*	15.7
Pelagophycus porra, stems	8.6	*Iridaea* spp	19.8
Nereocystis luetkeana, leaves	5.6		

FORMS OF SULPHUR IN KELP

Recent researches have emphasized the importance of sulphur as a constituent of plant tissues, and some investigators claim for this element an important rôle among the plant foods in which the soil may be deficient. Peterson (19) reviews the literature of the subject and gives tables of analyses showing the total content of sulphur and its forms in various plants considered to be exceptionally high in this constituent. Analyses of the kelp of California indicate much higher percentages of sulphur in many cases than those found in land plants of high sulphur content. It has been noted before that many marine algæ in other parts of the world contain considerable quantities of sulphur (6, Bd. 2, p. 820), but the proportion of sulphate to the total sulphur has not received attention. Table VI gives the percentages of total sulphur in various species of California kelps, and also the approximate division of the total sulphur content between organic and inorganic.

This was effected by leaching out all of the soluble sulphur and directly precipitating the solution with barium chlorid, using essentially the method of Folin. The sulphur precipitated directly by barium chlorid was subtracted from the total sulphur obtained by peroxid fusion and the difference regarded as organic sulphur. This was found to correspond approximately to the sulphur driven off on charring the sample.

TABLE VI.—*Percentage of sulphur in dried Pacific coast kelp*

No.	Material used.	Total sulphur.	Inorganic sulphur.	Organic sulphur.
1. 1	*Macrocystis pyrifera*, leaves	1. 25	0. 55	0. 70
1. 2	*Macrocystis pyrifera*, stems	. 82	. 38	. 44
2. 1	*Macrocystis pyrifera*, leaves	1. 14	. 46	. 68
2. 2	*Macrocystis pyrifera*, stems	. 72	. 36	. 36
15. 1	*Pelagophycus porra*, leaves	1. 03	. 49	. 54
15. 2	*Pelagophycus porra*, stems	. 71	. 28	. 43
29. 1	*Nereocystis luetkeana*, leaves	1. 27	. 82	. 45
29. 2	*Nereocystis luetkeana*, stems	. 45	. 14	. 31
42	*Egregia menziesii*	1. 17	. 32	. 85
43	*Laminaria andersonii*	2. 12	1. 07	1. 05
44	*Iridaea* spp.	8. 16	4. 52	3. 64

It will be noted that the leaves have a uniformly higher percentage of sulphur than the stems. For *Iridaea* spp. the amount of sulphur present is remarkably high. The exact nature of the organic combinations of sulphur is not known. Preliminary experiments indicate that the substances precipitated by alcohol contain a considerable proportion of organic sulphur; the protein sulphur may also be large. In order to test the presence of volatile sulphur compounds, steam distillations of kelp were made, in some cases from 10 per cent hydrochloric-acid

solutions, but no evidence of volatile sulphur could be obtained. Various samples of kelp were also boiled with alkali, but no tests for lead-blackening sulphur were apparent. The possible presence of volatile sulphur lost on drying has not yet been verified. Further work is planned to clear up these points.

FORMS OF IODIN IN KELP

Various researches have shown that many forms of marine life, such as the coral and the sponge, contain large amounts of iodin in combination with proteins or amino acids (9, 17, 33). By analogy it might be assumed that the 0.1 or 0.2 per cent of iodin present was also organically combined. Eschle (7) studied *Fucus vesiculosus* and *Laminaria digitata* with this in mind. He found that he could only extract 10 per cent of the iodin from the dried weed with boiling alcohol. From this and other extraction experiments he concluded that most of the iodin was organically combined.

Extractions of dried samples of Pacific coast kelps made in this laboratory indicate that nearly all the iodin is extractable by cold water or 90 per cent alcohol. From the aqueous solutions iodin may be set free by dilute potassium permanganate or potassium nitrite, which would lead to the inference that the iodin is present in ionic form. To determine whether the iodin could be completely extracted by water, a sample of dried and ground kelp was repeatedly digested and washed with warm water until the washings showed no further test for chlorin. The residue was found to still contain 5 per cent of the total quantity of iodin present. Another sample was treated in a similar manner, except that a very dilute alkali was used first for extraction. In this case the residue retained only a faint trace of iodin. It is possible that a small percentage of iodin is always present in organic combination, soluble in alkali, while a much larger amount exists as the iodid. Bromin is also found in the kelp, but only in one-fifth to one-tenth the quantity of iodin. The analysis of sea water shows a quite opposite condition, the amount of bromin largely exceeding that of iodin. There is a marked selective power in the kelp for iodin, although the exact function of this element is not known. Certainly the quantities of iodin retained by these plants are enormous as compared with the concentration in the sea water which bathes them. The selective action for potash is of course almost equally striking, but this difference is of interest; much of the potassium chlorid effloresces out as the plant dries, while no iodin is demonstrable in the effloresced salt. Many questions bearing on the essential or nonessential character of the various chemical elements present in kelp could be solved only by propagation in artificially controlled solutions. Such experiments would be of extreme interest, but would be difficult or impossible of execution.

ECONOMIC CONSIDERATIONS

FEEDING VALUE

The extensive use in Japan and Hawaii of certain seaweeds as articles of food has given rise to the suggestion that the giant kelp might be utilized for feeding man or animals. This question was discussed in a general way some years ago by Alsberg (4, p. 263–270), but he pointed out that practically no data regarding the composition of the kelps were available at that time. The chemical studies reported in the present paper make it evident that from the standpoint of nutrition the principal varieties of the California kelps could have but slight value. The carbohydrates are undoubtedly of a very resistant type, hydrolized with great difficulty, and their percentage utilization would necessarily be low. Saiki (20) has investigated this question for the carbohydrates of Irish moss (*Chondrus crispus*), several varieties of Japanese edible seaweeds, and agar-agar. Digestions were made with ptyalin, pancreatic amylase, and intestinal extract. In none of the cases was there any evidence of hydrolysis by the enzyms present. Feeding experiments on a human subject and on a dog gave very low coefficients of digestibility. This has been the import of many other experiments (16) in which pentosans, galactans, and similar carbohydrates have been investigated with reference to their nutritive value. It has never been shown that they are directly hydrolyzable by any of the enzyms of the digestive tract. Some value they unquestionably have because of bacterial decomposition, especially for animals of the ruminant type, but these resistant carbohydrates are at the best of low rank among feeding materials.

Analyses of the Pacific coast kelps show in some cases very appreciable percentages of nitrogen. If this were all in the form of utilizable proteins, it would make a very important addition to the feeding value, but it is doubtful whether such is the case. It has been shown earlier in this paper that a considerable portion of the nitrogen exists in the nonprotein form. Although the percentage of acid amid nitrogen is apparently very small, it would still be necessary to prove that the remainder of the soluble nitrogen was present in the form of suitably proportioned amino acids, before a high nutritive value could be assigned to the material. Furthermore, the nitrogenous compounds would undoubtedly be rendered less available because of the admixture of large percentages of highly resistant polysaccharids.

In order to recover the potash, it would be necessary to leach the kelp. Only the residue would ordinarily be considered for feeding purposes. Since much of the organic matter is soluble in water, the value of the residue would be still further decreased. Moreover, it is not believed that the kelp would produce a very palatable ration. Mr. F. W. Woll, of the University of California Division of Animal Industry, reports that cows

will not eat the leached or unleached fresh kelp unless it is well mixed with other feed.

In order to ascertain whether kelp might be preserved in the fresh state as a sort of silage, a sample of *Nereocystis luetkeana* was packed in an air-tight container and stored for three months. At the end of this period there was no indication of putrefaction. The acidity had increased slightly, the final percentage being 0.18 as lactic acid. The sample had become soft and "crumbly," but there was no formation of reducing substances or marked increase in soluble material.

UTILIZATION OF BY-PRODUCTS

Many uses were suggested by Stanford for the so-called algin. Various patents for the manufacture and application of this material were obtained (10-14). It was considered to be especially adapted for use in sizing papers and fabrics. That a substance of this nature might serve such a purpose is undoubtedly true, but that it would be commercially profitable is questionable. From a mechanical point of view the preparation of algin is difficult. The alkaline solution is extremely troublesome to filter, while the final product is very bulky, having only a very small proportion of dry matter. The dried material becomes very resistant to solvents. It would not be adapted to the preparation of spirit varnishes, since it is insoluble in alcohol, turpentine, and like solvents. It is true that an algin solution has a very high viscosity, but it does not follow that it possesses the properties of an adhesive, and such is, in fact, not the case.

Suggestions had been advanced that algin might serve for clarification of wines. Mr. W. V. Cruess, of the University of California Division of Enology, made several experiments to test this point. He found that the physical properties of the product did not well adapt it to the process of clarification. A further suggestion was to utilize the leached kelp in the manufacture of paper. It is difficult to understand how any of the usual types of paper could be prepared from a plant having such a low cellulose content. Redwood wastes and others of much greater possibilities are still to be utilized.

DESTRUCTIVE DISTILLATION

Balch and others have claimed that kelp might be destructively distilled and yield a profit. Balch (1) states that the volatile products from kelp, acetic acid, methyl alcohol, and tar "may be regarded as approximating in value those of beech wood." No experimental evidence is presented in support of this conclusion. In order to obtain data which would justify definite statements in regard to these points, distillation experiments were made in this laboratory. The apparatus used was an iron retort of about 1½ gallons' capacity, provided with a pyrometer and a suitable condenser. Distillation experiments made in this way

on a laboratory scale are open to the objection that they are not comparable with commercial practice. It was decided, therefore, to make control experiments under identical conditions, with materials of already ascertained value for purposes of destructive distillation. These distillations were made on oak sawdust and Douglas fir shavings. A large number of distillations were conducted under varying conditions, the results of which are recorded in Table VII.

TABLE VII.—*Comparative distillations of Pacific coast kelp, Douglas fir, and oak*

Run No.	Material used.	Quantity used.	Moisture.	Total distillate.	Total distillate less moisture.	Settled tar.	Charcoal.	Gas (by difference).	Total time of distillation.	Percentage of dry weight of material.		
										Acid as acetic.	Methyl alcohol (100 per cent).	Settled tar.
		Kg.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Hrs.*			
[a] 3	*Macrocystis pyrifera*	1	116	200	174	.....	465	245	.....	.....	.....	.....
4	....do	1	116	265	149	.....	470	265	.....	.....	.....	.....
5	....do	1	116	250	134	.....	480	270	.....	.....	.....	.....
[b] 6	....do	1	116	255	139	.....	465	280	.....	.....	.....	.....
7	....do	1	116	277	161	.....	475	248	.....	.....	.....	.....
8	....do	1	116	275	159	.....	510	215	.....	.....	.....	.....
[c] 9	....do	1	116	265	149	.....	500	235	.....	.....	.....	.....
10	....do	1	116	268	152	.....	480	252	.....	.....	.....	.....
11	....do	1	116	262	146	48	485	253	5	0.34	0.14	5.4
22	....do	1	116	283	167	41	512	205	8¼	.49	aver-	4.6
[d] 13	....do	1	116	277	161	60	472	251	4	.28	age.	6.8
[e] 14	....do	1	116	242	126	37	487	272	4	.28		4.2
[f] 15	....do	1	116	217	101	10	515	268	8	.....	.....	3.4
23	....do	1	116	277	161	60	485	238	4	.49	.20	6.8
[g] 24	....do	1	116	305	189	55	460	235	8	.58	.18	6.2
16	Douglas fir (*Pseudotsuga taxifolia*).	1	190	414	224	60	260	326	3	1.5	.7	7.0
17	....do	1	190	434	234	80	276	300	2½	.....	.7	10.0
25	....do	1	190	450	260	100	300	250	4	1.6	.....	12.0
[h] 18	Oak (*Quercus* spp.) sawdust	1	366	550	184	45	200	250	4	2.9	1.1	7.0
19	....do	1	313	424	111	60	230	346	3½	2.6	1.3	9.0
[i] 20	....do	1	313	412	99	37	215	373	4½	2.5	1.2	6.0

[a] Retort kept at red heat 7½ hours.
[b] Heated rapidly to red heat.
[c] Very slow distillation at low heat, occupying 12 hours.
[d] Distillation started 7.20 p. m.; 7.45 p. m., pyrometer 120° C.; 9 p. m., 190° C.; 9.30 p. m., 200° C.; 3.10 p. m., 220° C.; 4.05 p. m., 240° C. (gases slightly combustible); 4.30 p. m., 360° C. (gas burns steadily); 4.50 p. m., 420° C.; 5.10 p. m., 500° C. (no more distillate).
[e] Distillation started 8.30 a. m.; 9.15 a. m., 160° C. (large watery distillate); 9.30 a. m., 200° C.; 10.35 a. m., 220° C.; 11.15 a. m., 280° C. (gases slightly combustible); 11.30 a. m., 310° C. (distillate oily; gas burns); 12.25 p. m., 500° C.
[f] Distillation started 9 a. m.; 9.30 a. m., 140° C.; 9.50 a. m., 170° C.; 10.45 a. m., 190° C.; 12 m., 200° C.; 2 p. m., 220° C.; 2.15 p. m., 290° C.; 3.20 p. m., 390° C.; 4.30 p. m., 400° C.
[g] Distillation started 8.35 a. m.; 9.10 a. m., 160° C.; 11 a. m., 230° C.; 12.45 p. m., 250° C.; 1 p. m., 300° C.; 2.45 p. m., 440° C.; 4.45 p. m., 530° C.
[h] Distillation started 8.35 a. m.; 9.05 a. m., 170° C. (gases burn); 9.15 a. m., 200° C.; 10.05 a. m., 270° C.; 11.50 a. m., 290° C.; 12.30 p. m., 530° C.
[i] Distillation started 1.05 p. m.; 1.40 p. m., 160° C.; 2.40 p. m., 200° C.; 3.20 p. m., 250° C.; 5.25 p. m., 500° C.

Inspection of this table shows that the distillates from the kelp, judged by their content of acetic acid and alcohol, had a value of only one-fifth to one-tenth that of the oak and fir distillates, a value so slight as to preclude any profitable recovery of the products. The yields for oak and fir approximate those obtained in larger experiments on similar materials, and it is very probable, therefore, that the general comparisons with kelp would hold even in distillations on a commercial scale.

The distillates obtained from the kelp were watery in appearance and had a very slightly acid reaction to litmus, although they contained considerable amounts of basic substances. By the Kjeldahl method 3.2 gm. of nitrogen was found in the total distillate from 1 kg. of dried kelp. The tar oils obtained with the distillate floated on the surface, having a specific gravity of 0.984. Their percentage varies from 4 to 7 on the basis of the dry kelp. The gases evolved from the kelp differed from those of the oak and fir in not being combustible during any of the earlier stages of the distillation. The charcoal residue in the retort was soft and of dull-gray color. Leaching experiments indicate that most of the potash may be recovered from the char as a high-grade product.

Further details of the above work will be considered in a later article.

SUMMARY

(1) After a brief résumé of the literature, the general chemical composition of the principal species of Pacific coast kelps is discussed. An extended series of analyses is presented, with experimental data concerning the nature of algin and other carbohydrate bodies present.

(2) The forms of nitrogen in the kelp are considered. Much of the nitrogen is found to be present in nonprotein form.

(3) Experiments are reported on the form of the iodin, of which only a small proportion is believed to be organically combined.

(4) The high content of organic sulphur in the kelp is noted and a table of analyses given.

(5) The economic phases are discussed with reference to feeding value and utilization of organic by-products. The results indicate only slight possibilities of commercial value in these directions.

(6) Comparative laboratory experiments on the destructive distillation of kelp are presented, and the conclusion is reached that kelp distillates are of no practical importance.

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SOURCES OF THE EARLY INFECTIONS OF APPLE BITTER-ROT[1]

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INTRODUCTION

Notwithstanding the excellent work which has been done by previous investigators, the problem of determining the sources from which the early infections of the apple bitter-rot fungus (*Glomerella cingulata*) may arise has never been worked out with completeness sufficient to account for the heavy initial damage sometimes caused by this disease. Often bitter-rot will break out suddenly, and every apple (*Malus* spp.) in an orchard will be affected in an incredibly short time. In the Ozark region of Arkansas during the season of 1914 the crops of six of the orchards under the writer's observation were within two weeks almost entirely ruined by bitter-rot. Nearly all the apples on these trees were infected comparatively early in the season—i. e., about July 1—a large majority of them having from 50 to 100 points of infection. The suddenness of the appearance of the disease and the almost simultaneous infection of the fruit over the whole orchard strongly indicated that practically all the rotten spots were caused by spores which had washed down from primary sources of infection. In one orchard soon after the disease broke out nearly every apple was found to be affected with the small blister-like spots characteristic of the early stages of the disease (Pl. VII, fig. 1). These spots had not yet developed far enough to produce acervuli and were evidently due to infection by spores from overwintering or primary sources.

The later infections are, of course, easily accounted for because the fungus forms acervuli in the rot areas of the earlier infections and from these the spores may be washed by rain or carried by insects to sound apples, which, if conditions are favorable, may become diseased and in turn become sources of infection. Thus, we may have primary sources of infection, which may continue to act as such throughout the season, and secondary sources of infection, consisting of the diseased fruits of the current season.

The primary sources of infection therefore become of great importance in the control of the disease, especially when they are present in great

[1] A brief but incomplete report of these investigations was made before the Northwest Arkansas Fruit Growers Society in July, 1914. (Roberts, J. W. The sources of apple bitter-rot infection, and control. *In* Ozark Fruit and Farms, v. 5, no. 2, p. 3. August, 1914.)

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abundance. Under ordinary conditions, even with the weather favorable to the development of the fungus, bitter-rot may be practically prevented by spraying with Bordeaux mixture. When, however, in addition to favorable weather conditions, the primary sources of infection are as abundant as they are in some of the orchards of the Ozarks and probably other sections in which the disease is prevalent, spraying alone will, by reducing the number of infections, only retard rather than prevent. To gain success by spraying it would be necessary to keep the entire surface of every apple continually covered with Bordeaux mixture, a physical impossibility.

HISTORICAL REVIEW OF LITERATURE

The early infections of bitter-rot have been explained somewhat differently by different writers, all of whom doubtless give correct explanations for the particular regions or orchards in which their investigations were made. None of these writers, however, has made his investigations complete enough to account for the numerous early infections which sometimes occur.

Simpson, of Illinois, discovered that primary infections of bitter-rot were associated with a certain type of twig canker (Burrill and Blair, 1902, p. 355; Von Schrenk and Spaulding, 1903, pp. 30–31).[1]

Burrill and Blair (1902, p. 356) discuss the fungus in relation to early infections as follows:

> It therefore became evident that the disease on apples could come from these spots on the branches, and everything now goes to show that except in the few cases that the rot mummies hang over on the trees, the first or early infection comes solely from these limb cankers. * * * It now seems to be commonly true that the cankers are few in number, at least upon the kinds of trees ordinarily planted in Illinois and not over 15 years of age.

Clinton (1902) expresses the belief that the early infections come from the ascogenous stage of the fungus, developing in mummies of the preceding year.

Hasselbring found that in mummied apples kept out of doors the fungus ordinarily retains its vitality in a dormant state in the winter and in May or later under proper conditions again begins to produce conidia (Burrill and Blair, 1902, p. 354).

Von Schrenk and Spaulding (1903, pp. 37–38) showed by inoculations that the limb cankers discovered by Simpson were actually caused by the bitter-rot fungus. They also state:

> The apparently erratic behavior of the bitter rot can be explained in part since the discovery of the canker stage of the fungus. After its introduction into an orchard or on one tree the fungus attacks one or more branches, probably early in the summer, and produces a canker. The next year the spores from this canker will be washed down on the ripening fruit by a rain. The water is sprayed from the branch on which the canker is situated to the lower branches in the form of a cone, and one or more spores will probably fall on every apple within such a cone. The presence of the

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 64.

winter stage of the fungus will explain why the rot is apt to recur on the trees affected the year before with the bitter rot, and also why the disease should first appear on such trees. The cankers produce spores early in the season, and from the trees which have cankers the disease spreads to neighboring trees. * * * It now seems probable that the mummies play a comparatively small part in serving as distributing points for spores from year to year.

Alwood (1902, pp. 264–265, 270), after extensive investigations of the disease in Virginia, states:

Diligent search of the limbs failed to show any bitter-rot cankers on these [susceptible] varieties mentioned. * * * In no instance have we been able to find the presence of the bitter-rot fungus on the limbs or trunks of apple or pear, though we have especially watched for its occurrence since the appearance of the publication cited [Burrill and Blair]. * * * It appears to be well established that the mummied fruits hanging to the trees and the rotted fruits upon the soil constitute in a large measure the source of the annually recurring infection.

Scott (1906, p. 12), after investigating the disease in Virginia, agrees with Alwood in that he considers mummies as the chief sources of infection. He states:

The results lead to the conclusion that the overwintering mummies hanging on the trees constitute the chief source of infection, at least in this particular region. In the majority of cases examined a mummy could be found in the upper portion of the infected area, but in no case was there found associated with such outbreaks any cankers that could be identified as bitter-rot cankers.

Shear and Wood (1913, p. 76) obtained *Glomerella cingulata* from a great variety of plants, and it is possible that in some cases early infections may come from hosts other than the apple.

INVESTIGATIONS IN THE OZARKS

The large number of primary infections in some of the orchards of the Ozarks from which mummies had been practically all removed and in which the bitter-rot cankers as described by previous writers were few or wanting led the writer to undertake to discover from what sources the early infections were arising in such serious abundance. It was impossible to believe that mummies and bitter-rot cankers, so few in themselves, could be the sole harboring places of a fungus which could cause from 10 to 200 rotten spots to appear on nearly every apple on large, heavily laden trees.

In this region cankers and dead areas on limbs, due to various causes, are very abundant. The Illinois apple-tree canker, caused by *Nummularia discreta*, is a very prevalent and serious disease. Cankers and dead areas due to *Bacillus amylovorus*, *Phyllosticta solitaria*, and various physiological and mechanical causes are also quite numerous. In some of the orchards it is almost impossible to find a branch or twig which does not show several of the cankers caused by *P. solitaria*.

Considering these cankers as possible sources of early infections, all cankers and dead wood, in so far as practicable, were removed in the

spring of 1914 from parts of two orchards in which the disease in previous years had not proved amenable to spraying. In one of these orchards the part from which the cankers had been eradicated was the section of the orchard in which during previous years bitter-rot had been most destructive. The fruit of this part came through the season practically free from rot, while about 50 per cent of the fruit of the part from which the cankers were not removed was destroyed by the disease. Both of these parts were sprayed four times at intervals of two weeks, beginning June 15. In the second orchard the fruit of neither plot was sprayed, and all of it eventually rotted. In the plot in which the cankers were allowed to remain every apple was infected by the middle of July, whereas in the plot from which the cankers and dead wood had been removed destruction was not complete until two months later. Every apple in the untreated plot was evidently infected from primary sources, since there were as yet no secondary sources. While an occasional apple was found which showed only 1 infection, nearly every one of them showed at least 50 and many of them were literally covered with the tiny, blister-like spots. In the treated plot early infections were considerably less in number, and a majority of the fruit was free from them. Later in the season, however, all fruit that had escaped the early infections finally became infected from secondary sources.

On May 15 a cankered limb from the second orchard was brought into the laboratory and kept in a moist chamber for 24 hours. This canker resembled in every way the limb cankers as described and figured by Burrill and Blair and Von Schrenk and Spaulding (Pl. VII, fig. 2). It was a black, sunken, oval area, with many slight rifts or cracks in the bark through which, after the limb had remained in a moist chamber for 24 hours, an abundance of the characteristic pink acervuli appeared. Near the center of this canker was a small dead spur through which infection probably took place.

Cankers resembling in every way published descriptions and figures of bitter-rot cankers were also collected on June 3 and many times thereafter.

During the month of May there was collected from one of these orchards a limb which was badly infected with *Nummularia discreta*, and while spore masses of *Glomerella cingulata* were not abundant on it, yet enough were present to make it a dangerous source of infection under proper conditions. Later in the season spores were many times obtained from Nummularia cankers.

Spore masses of the fungus were also found on a long dead, well-delimited part of a large limb in one of the orchards before mentioned. This area was about 8 cm. wide and about 2 meters long. Wide cracks along its margins sharply separated it from the living part of the limb (Pl. VII, fig. 3). Such strips of dead tissue are usually assigned to injury by freezing or to the death of roots on one side of the tree. While

the spores from this source were comparatively few, they were, nevertheless, sufficient to give the disease a good start. In the same orchard an occasional fruit spur was found from which spores were somewhat sparingly produced after it had been kept in a moist chamber for from 24 to 48 hours. The part from which the fungus was obtained was the dead tip on which the fruit of the preceding year had been borne.

In at least two well-authenticated cases acervuli were found on the injured parts of small limbs which had been nearly girdled by the organism of pear-blight (*Bacillus amylovorus*). These cankers had been caused by the blight organism infecting and killing a small twig and going thence into the tissues of the limb at the base of the twig (Pl. VII, fig. 4).

In one orchard which had been badly infected for years it was possible to find the fungus on almost any sort of cankered or injured limb. From directly above a mass of badly infected apples still hanging on the tree, a small limb having a long dead area just beginning to be healed over through the formation of callus was removed (Pl. VII, fig. 5). This injury had apparently been brought about by mechanical means; probably the limb had been severely scraped by the tower of a power sprayer or by a wagon box or hayrack, all of which were accustomed to pass through the orchard at frequent intervals. After a short time in a moist chamber acervuli appeared from beneath narrow strips of dead bark which lay near the angle formed by the dead area and the overlapping callus. This injured area was 19 cm. long and 4 mm. wide and contained innumerable acervuli. That this mechanically injured limb had served as a source of direct infection was indicated by the fact that there were a large number of badly infected fruits just below it, whereas all the apples above it were sound. No other possible sources of infection were present.

This orchard was also badly infected by the apple-blotch fungus (*Phyllosticta solitaria*). The cankers caused by this organism were quite numerous on the smaller limbs and branches, especially in the older part of the orchard which had been practically abandoned. In connection with some work with the apple-blotch fungus the writer had occasion to scrape from these cankers (Pl. VII, fig. 6) spore-bearing pycnidia of *Phyllosticta solitaria*, which, after being crushed, were placed in Van Tieghem cells so that spore germination might be observed. In many cases bits of bark were accidentally carried into the Van Tieghem cells along with the pycnidia and spores of the blotch fungus. Repeatedly during the month of May and later as well there grew out from these small bits of bark hypæ which produced spores (conidia) of the bitter-rot fungus. Cultures from these spores produced the characteristic acervuli and conidia and often the perithecia and ascospores of *Glomerella cingulata*.

Masses of spores were also obtained many times from mummies; and where mummies are present, they undoubtedly are important sources

of infection. In many of the badly infected orchards, however, they had been removed both from the trees and from the ground.

The fungus from all the sources mentioned was positively identified as *Glomerella cingulata* not only by microscopical observations and spore measurements but by spore germination and growth on artificial media as compared with germination and growth from spores from actual cases of bitter-rot of the fruit. Also in all cases sound, sterile apples were inoculated with spores from pure cultures, the typical bitter-rot disease was brought about, and the fungus reisolated. Thus, the spores from every source of infection discussed as such in this paper were proved to be those of the bitter-rot fungus.

SUMMARY

(1) Previous writers have shown that the apple bitter-rot fungus (*Glomerella cingulata*) may pass the winter in mummied apples of the preceding year and in bitter-rot cankers from which the early infections of the following season may come. Other plants also may be possible sources of infection.

(2) The writer has shown that in apple orchards where the infections have been severe the fungus may winter over on almost any cankered or dead parts of the tree, including the Illinois apple-tree canker due to *Nummularia discreta;* dead tips of fruit spurs; dead parts of limbs due to injury by freezing or to death of roots; branches injured by mechanical means; cankers caused by the pear-blight organism (*Bacillus amylovorus*); twig cankers caused by the apple-blotch fungus (*Phyllosticta solitaria*).

(3) Eradication of cankers greatly reduced the number of early infections of the disease, though removal of all small dead parts, such as dead tips of fruit spurs and small mechanically injured places, is, of course, practically impossible.

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PLATE VII

Fig. 1.—Givens apple having numerous small blister-like infections of bitter-rot.

Fig. 2.—Bitter-rot canker from a Jonathan apple tree.

Fig. 3.—Part of a branch of a Givens apple tree which had been injured probably by freezing. Acervuli of the bitter-rot fungus were obtained from the dead part of this branch.

Fig. 4.—Apple branch showing blighted area on which acervuli of the bitter-rot fungus were found.

Fig. 5.—Mechanically injured branch of a Missouri Pippin apple tree. Acervuli of the bitter-rot fungus were found about the margins of the injured part.

Fig. 6.—Branch of Missouri Pippin apple tree affected with apple-blotch. The bitter-rot fungus was found to be wintering over in blotch cankers.


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A BACTERIOLOGICAL STUDY OF METHODS FOR THE DISINFECTION OF HIDES INFECTED WITH ANTHRAX SPORES

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INTRODUCTION

The number of hides and skins imported into this country each year amounts to many millions. Since these come to us from all quarters of the globe, it is evident that there is danger that they will bring with them infectious material which may cause disease among animals and human beings.

On account of the great resisting power of the anthrax spore, hides and skins imported from countries where anthrax is prevalent are regarded as especially dangerous; and inasmuch as methods of disinfection which will destroy the anthrax spore may be expected to kill other organisms with ease, considerable attention has been devoted to the problem of securing a disinfectant that will destroy the anthrax spores without damaging the hides and skins. Among the numerous processes which have been suggested, that devised in 1910 by Seymour-Jones (16)[2] has attracted much attention, while more recently the Schattenfroh (12) method has been declared by various investigators to be equally efficient and by some even more so.

As Eurich (1, 2), Ponder (9, 10), Seymour-Jones (16), and others have pointed out, the spores of anthrax are found chiefly in connection with blood stains, and as these, together with other material with which the spores are likely to be associated, are colloidal in nature, the problem, as Seymour-Jones expresses it, is to get at the anthrax spore "when imbedded in a gelatinous, albuminous, or other colloidal body without injury to the material or fabric to be disinfected."

OUTLINE OF SEYMOUR-JONES AND SCHATTENFROH METHODS OF DISINFECTION

Seymour-Jones (16) proposes to attain the desired result by the use of mercuric chlorid and formic acid. He holds that the acid causes the hide and the various associated colloidal substances to swell, absorb water, and become soft and tender, thus furnishing favorable conditions for the action of the mercuric chlorid. Under these conditions he con-

[1] The writer desires to express his obligations to Mr. F. P. Veitch, Chemist in Charge, Leather and Paper Laboratory, Bureau of Chemistry, for the work done under his direction in tanning pieces of disinfected hide, and to Dr. E. C. Schroeder, Superintendent, Bureau of Animal Industry Experiment Station, for facilities afforded in carrying out the experimental work upon animals.

[2] Reference is made by number to "Literature cited," p. 91–92.

siders a dilute solution of mercuric chlorid sufficient for disinfection. After disinfection hides are transferred to a saturated solution of common salt, whereby, it is claimed, they will be shrunk and brought to the "wet salted" state. The dilutions recommended are mercuric chlorid, 1 part in 5,000, with 1 per cent of formic acid; and the time of exposure to the disinfectant, 24 hours.

One of the first workers to investigate the Seymour-Jones process was C. W. Ponder (9, 10). He found that artificially infected pieces of hide were not disinfected in 24 hours by a solution of mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, in 4 cases out of 10 and concluded that the effective dilution of mercuric chlorid lay between 1 to 1,000 and 1 to 5,000. In spite of these results he recommends the service use of mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, on the ground that his tests were made more rigorous than was necessary to meet the conditions obtaining in actual routine disinfection. It is worthy of note that he made no attempt to neutralize the disinfectant before testing the results by cultures and by inoculation of animals. Moegle (7) and Schnürer (13) have also reported favorable results with the Seymour-Jones method.

The investigations of Ševčík (14) controvert all these favorable results. By neutralizing the disinfectant with sodium sulphid he was able to obtain living and virulent anthrax bacilli from spores treated with very strong dilutions of mercuric chlorid and formic acid, even when the time of exposure was extended to a number of days. Judging from his published results, it would require a dilution of mercuric chlorid, 1 to 500, plus 1 per cent of formic acid, to destroy anthrax spores in 24 hours. The use of sodium sulphid in this manner does not seem unreasonable, since, as a matter of fact, many tanners use this substance for dehairing hides. Hilgermann and Marmann (4) have obtained similar results with the Seymour-Jones method, using ammonium sulphid as a neutralizing agent.

Another method for the disinfection of hides which has recently come into prominence is the method of Prof. Schattenfroh (12), which depends upon the use of hydrochloric acid and sodium chlorid. The amounts recommended for use at room temperature are 2 per cent of the acid and 10 per cent of the salt, with a 48-hour exposure. At higher temperatures weaker dilutions may be employed.

Gegenbauer and Reichel (3) have carried on an extensive research with this method and report entirely favorable results. They state that they consider the Seymour-Jones method inefficient on account of the low concentration of mercuric chlorid and also object to its use because of the discoloration by mercuric sulphid when sodium sulphid is used for dehairing. Their statements regarding the Seymour-Jones method appear to be based upon experimental work not yet published. The favorable results obtained by Gegenbauer and Reichel with the Schattenfroh method are confirmed by the favorable results obtained by Hilger-

mann and Marmann (4) as the result of comparative experiments with the Seymour-Jones and Schattenfroh methods.

Ševčík's comparison (14) of the two methods is interesting, but is not fair to the Schattenfroh method, as he admits, because of the use of solutions based on the percentage of "hydrochloric acid" rather than on the percentage of hydrochloric-acid gas.

EXPERIMENTAL WORK ON GERMICIDAL EFFICIENCY OF DISINFECTANTS

The experimental work was undertaken primarily with a view to determining the value of the Seymour-Jones method, and for that reason this paper deals largely with work done with that method, although some attention was paid to others, especially the Schattenfroh method.

In the absence of a supply of naturally infected hides it was necessary to make the experiments upon pure cultures and artificially infected pieces of hide. Although Ševčík (14) states that naturally infected hides are better for test preparations than those artificially infected, it does not seem that the difference is as great as he claims. Certainly his results with naturally infected hides, where the disinfectant was not neutralized, correspond very closely to results obtained by Ponder (9, 10) with artificially infected hides.

EXPERIMENTAL PROCEDURE

For preliminary work the Hill "rod" method (5) seemed best adapted; so this was used, with some modifications. The method as modified is as follows: Glass rods three-sixteenths of an inch in diameter and 8 inches long are etched at one end, the etched portion being about 1 inch long. Cotton is wrapped about the rods near the end not etched and the rods thrust into test tubes so as to engage the cotton in the mouth of the tube. The tubes containing the rods are sterilized by dry heat (150° C.) for one hour or more. In making tests the rods are removed and the etched portion dipped into a suspension made from a culture of the organism employed and this allowed to dry on the rod.

Rods so infected are transferred to test tubes containing the disinfectant to be tested and there exposed to its action for varying lengths of time. After exposure the rods are washed with sterile water in order to remove traces of the disinfectant and are then transferred to tubes containing bouillon or agar, which are incubated for at least 48 hours at 37.5° C. The suspension used in infecting the rods is made from the surface growth on an agar tube by rubbing up in several cubic centimeters of sterile water enough of the growth to give a suspension of approximately the same density as a 24-hour bouillon culture of *Bacillus typhosus*. For a non-spore-bearing organism the culture should be 24 hours old, while for spore-bearing organisms cultures 1 to 2 weeks old are usually the most suitable.

In making tests with disinfectants containing mercury it is advisable to dip the rods into a saturated solution of hydrogen sulphid or an aqueous solution of some sulphid before placing them in subculture tubes. In this connection it should be mentioned that media of acid reaction have been found to exert an inhibitory action upon the growth of *Bacillus anthracis* after exposure to disinfectants. For that reason the media employed in these experiments have been neutral or slightly alkaline.

A considerable number of tests by the rod method were made with organic matter added to the disinfectant. This was done by removing a certain portion of the total volume of disinfectant and substituting a like amount of defibrinated blood.

Inasmuch as the use of a solution of sodium chlorid did not seem essential in experiments upon "naked" anthrax spores, since this salt is said by Seymour-Jones to be used in his method to reduce the swelling of the hides caused by formic acid, a common salt solution was not used in the "rod" method experiments.

MERCURIC CHLORID AND FORMIC ACID

I. EXPERIMENTS BY ROD METHOD, USING BUREAU OF ANIMAL INDUSTRY STRAIN OF BACILLUS ANTHRACIS

These experiments were designed to show the germicidal efficiency of mercuric chlorid ($HgCl_2$) with and without formic acid (CH_2O_2) and with and without the addition of defibrinated blood.

In experiment 1 (Table I) the rods were infected by using an agar culture 2 weeks old for making the spore suspension. Microscopical examination of the suspension showed that plenty of spores were present. Each rod was exposed to 5 c. c. of disinfectant for 24 hours and was then washed in 20 c. c. of hydrogen-sulphid solution or sterile distilled water.

The rods were then transferred to subculture tubes of exactly neutral broth and incubated at 37.5° C. for three days.

TABLE I.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, and of phenol by the rod method, without addition of organic matter* [a]

EXPERIMENT 1

Rod No.	Disinfectant (5 c. c.) and dilution.	Time of exposure	Results after incubation for—		
			18 hours.	1 day.	3 days.
		Hours.			
1	Mercuric chlorid (1:5,000)	24	No growth	Growth	Strong growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	....do	No growth	No growth.
3	Control rod	(b)	Strong growth	Strong growth	Strong growth.
4	Mercuric chlorid (1:5,000)	24	No growth	No growth	No growth.
5	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	....do	....do	Do.
6	Control rod	(b)	Strong growth	Strong growth	Strong growth.
7	Phenol (5 per cent)	24	No growth	Growth	Do.
8	Do	48	....do	....do	Do.

[a] Rods 1, 2, and 3 washed with hydrogen-sulphid solution, and Nos. 4, 5, 6, 7, and 8 with sterile water.
[b] Not exposed.

The results of the above experiment indicate that mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, is efficient where mercuric chlorid alone is not and that the hydrogen-sulphid solution should be used to neutralize the disinfectant before putting the rods into subculture tubes. The result after 48 hours' exposure to 5 per cent of phenol indicates the resisting power of the anthrax spores. The next experiment consisted of short exposures with the addition of defibrinated blood. This experiment was intended to test the efficiency of the method of disinfecting hides prescribed in Circular No. 23 of the Treasury Department, which consisted in immersion of hides for half an hour in a solution of mercuric chlorid, 1 to 1,000.

The technique was similar to that described for experiment 1, except that all rods were washed with hydrogen-sulphid solution and defibrinated blood was added so as to make up 10 per cent of the volume of the disinfectant in each tube. The results are given in Table II, experiment 2.

Experiment 2 indicates that anthrax spores are not destroyed in the presence of defibrinated blood by mercuric chlorid, 1 to 1,000, without formic acid, or by mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, even with an exposure of two hours.

An experiment with stronger dilutions of mercuric chlorid plus formic acid was now tried. Defibrinated blood was added in the proportion of 10 per cent of the total volume. (See Table II, experiment 3.)

TABLE II.—*Germicidal efficiency of mercuric chlorid, with or without formic acid, by the rod method, with the addition of defibrinated blood* [a]

EXPERIMENT 2

Rod No.	Disinfectant (5 c. c.) and dilution.	Time of exposure.	Result
		Hours.	
1	Mercuric chlorid (1:1,000)	¼	Growth.
2	Do	½	Do.
3	Do	1	Do
4	Do	2	Do.
5	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	¼	Do
6	Do	½	Do.
7	Do	1	Do.
8	Do	2	Do.
9	Control rod	(b)	Do.

EXPERIMENT 3

1	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	¼	No growth.
2	Do	½	Do.
3	Do	1	Do.
4	Do	2	Do.
5	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	¼	Do.
6	Do	½	Do.
7	Do	1	Do.
8	Do	2	Do.
9	Control rod	(b)	Growth.

EXPERIMENT 4

1	Mercuric chlorid (1:1,000)	24	No growth.
2	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Do
3	Control rod	(b)	Growth.

[a] Subculture tubes incubated one week. Rods washed with hydrogen-sulphid solution.
[b] Not exposed.

Returning to conditions more closely resembling the Seymour-Jones method, experiment 4 was carried out with a 24-hour exposure to the disinfectant plus 10 per cent of defibrinated blood. The results are given in Table II, experiment 4.

The results of the preceding experiments indicated that in the presence of 10 per cent of defibrinated blood anthrax spores are not destroyed in 2 hours by mercuric chlorid, 1 to 1,000, without formic acid, nor by mercuric chlorid, 1 to 5,000, with 1 per cent of formic acid, but that they are destroyed by mercuric chlorid, 1 to 2,000, with 1 per cent of formic acid, under the same conditions. On the other hand, anthrax spores are destroyed by mercuric chlorid, 1 to 1,000, without formic acid, and by mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, even in the presence of defibrinated blood, when the time of exposure is 24 hours.

On account of the greatly increased germicidal power of mercuric chlorid in the presence of formic acid observed in the foregoing preliminary experiments, it was deemed advisable to test the germicidal power of mercuric chlorid and formic acid against anthrax spores dried upon pieces of hide. The Bureau of Animal Industry (B. A. I.) strain of *Bacillus anthracis*, which was employed in the previously described "rod" method experiments, was used in infecting the pieces of hide.

The results of these experiments, both by cultural methods and by inoculation of animals, were entirely unsatisfactory, the reason for this being apparently that the B. A. I. strain of *Bacillus anthracis* produced spores of comparatively low virulence and low vitality.

For this reason a culture of an entirely different strain of *Bacillus anthracis* was obtained from the Army Medical School (A. M. S.) through the courtesy of Capt. Craig, and spores of this strain were used in all further experiments. Experiments were made with "naked" spores by the "rod" method and with spores dried upon pieces of hide. As the subsequent records of these experiments will show, the spores of the A. M. S. strain were found to be very much more virulent and resistant to the action of disinfectants, drying, etc., than those of the B. A. I. strain.

II. EXPERIMENTS BY ROD METHOD, USING ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS

The technique of these experiments was exactly the same as for those with the B. A. I. strain, except that the quantity of disinfectant per rod was made 10 c. c. instead of 5 c. c.

Experiment 5 (Table III) indicates that mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, is able to destroy anthrax spores of the A. M. S. strain in 24 hours when no organic matter is added.

TABLE III.—*Germicidal efficiency of mercuric chlorid and formic acid by the rod method, without addition of organic matter*

EXPERIMENT 5[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure	Result.
		Hours.	
1	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	No growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	24	Do.
3	Control rod	(b)	Growth.

[a] Incubated 5 days. Hydrogen-sulphid solution used for neutralization of mercury.
[b] Not exposed.

Experiment 6, on the other hand, indicates that with an addition of defibrinated blood mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, is not able to kill spores of the A. M. S. strain in 24 hours (Table IV).

TABLE IV.—*Germicidal efficiency of mercuric chlorid, with or without formic acid, by the rod method, with and without addition of organic matter*

EXPERIMENT 6[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Quantity of blood added.	Time of exposure.	Result
		C. c.	*Hours.*	
1	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	None.	24	No growth.
2	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	1	24	Growth.
3	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	None.	24	No growth
4	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	1	24	Growth.
5	Mercuric chlorid (1:7,000)	None.	24	No growth
6	Mercuric chlorid (1:1,000)	1	24	Growth.
7	Control rod		(b)	Do.

[a] Incubated 3 days. Saturated aqueous solution of hydrogen sulphid used for neutralization of disinfectant.
[b] Not exposed.

In another experiment with various dilutions (Table V, experiment 7) there were 9 c. c. of disinfectant plus 1 c. c. of defibrinated blood in each tube.

Experiment 7 was repeated with the result given in Table V, experiment 8.

In experiment 9 the technique was the same as for experiment 8, the age of the culture being approximately the same (Table V, experiment 9).

TABLE V.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, by the rod method,[a] with addition of defibrinated blood*

EXPERIMENT 7[b]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	No growth.
2	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Growth.
3	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)	24	Do.
5	Control rod	(c)	Do.

[a] Hydrogen-sulphid solution used to neutralize disinfectant.
[b] The quantity of disinfectant used in experiments 7, 8, and 9 included 1 c. c. of defibrinated blood.
[c] Not exposed.

TABLE V.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, by the rod method, with addition of defibrinated blood*—Continued.

EXPERIMENT 8 [a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
1	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Growth.
2	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
3	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)	24	Do.
5	Control rod	(b)	Do.

EXPERIMENT 9 [a]

1	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	No growth
2	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Growth.
3	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)	24	Do.
5	Control rod	(b)	Do.

[a] The quantity of disinfectant used in experiments 7, 8, and 9 included 1 c. c. of defibrinated blood.
[b] Not exposed.

The discrepancy between the results of experiment 7 and those of experiments 8 and 9 appeared to be due to the use of a culture only 7 days old for making the spore suspension used in experiment 7, while the cultures used in experiments 8 and 9 were 17 and 22 days old, respectively.

In all these experiments spore suspensions were examined microscopically to make certain that plenty of spores were present, and it was noted that where cultures were less than 10 days old the suspensions generally contained a greater number of bacilli in relation to the spores than suspensions made from cultures 2 to 3 weeks old. The older cultures were therefore better adapted for this work.

The results of these experiments and a number of other similar experiments indicated that the A. M. S. strain of *Bacillus anthracis* was much more vigorous than the B. A. I. strain, which was used in experiments 1 to 4, and consequently was better suited for the purpose of this work. The following experiments, in which pieces of infected hide were employed, were therefore carried on with spores of the A. M. S. strain.

III. EXPERIMENTS UPON PIECES OF HIDE INFECTED WITH SPORES OF THE ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS WITHOUT NEUTRALIZATION OF DISINFECTANT

Some of the pieces of hide were prepared by a method essentially the same as that described by C. W. Ponder (9, 10), the details being as follows: The test preparations were made by cutting out pieces of hide so that each piece weighed about 2½ gm. Blood was drawn from the ear of a rabbit and a good-sized drop allowed to fall on the center of the hair side of each piece. Before clotting occurred a loopful of a suspension of anthrax spores was mixed thoroughly into the drop of blood.

The loop used was 3 cm. in diameter of 23-gauge platinum wire. The preparations so made were dried in the incubator 23 hours and then kept at room temperature until used.

In view of statements made by Otsuki (8) that spores of anthrax are injured by drying at 37.5° C., and that the best method of preparation is by drying them at 10° C., another lot of test preparations of hide was made as follows: Pieces of hide were cut to weigh about 2½ gm. On each piece a good-sized drop of blood from a rabbit's ear was allowed to fall and into this was mixed a loopful of a suspension of anthrax spores. This suspension was prepared by rubbing up in sterile water enough of the surface growth from a 15-day agar culture to give a suspension approximately equal in density to a 24-hour bouillon culture of *Bacillus typhosus*. These pieces of hide were placed in Petri dishes with raised covers and were dried for three days in a desiccator over sulphuric acid at a temperature of 10° C. and in a vacuum equal to about 6 cm. of mercury.

Guinea pigs were inoculated with clots from pieces of hide dried by each method. In neither case were the spores found to possess sufficient vitality to infect the animals, and it seemed evident that the methods of preparation had in some way attenuated the virulence of the spores. In view of the statement made by Roos (11) that rabbit blood is bactericidal for anthrax bacilli, while guinea pig blood is not, it seemed that the lack of virulence might be due to the use of rabbit blood. Therefore new pieces of hide were prepared, using blood from a guinea pig instead of rabbit blood as before. The pieces of hide were dried for 24 hours at 37.5° C. and then kept several days at room temperature in a dark closet. The lower drying temperature was used in later experiments. The spores in these test preparations were found to be virulent for guinea pigs, although less virulent than the original A. M. S. culture when tested shortly after it was received.

The virulence of the cultures was therefore raised by successive inoculations until a culture was obtained which killed a guinea pig in about 36 hours after subcutaneous inoculation. This culture was then employed in preparing test pieces of hide by the method above described, guinea-pig blood being used and the pieces being dried at 37.5° C. The pieces of hide so prepared were subjected to the following tests:

Each piece of hide was exposed to 25 c. c. of disinfectant for 24 hours and then soaked in 25 c. c. of saturated salt solution for 24 hours. At the end of that time the clots were scraped off and inoculated into guinea pigs. The results are given in Table VI.

TABLE VI.—*Inoculation of guinea pigs with clots from pieces of hide*

EXPERIMENT 10

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots inoculated.	Result of inoculation.
		Hours.		
23237	Mercuric chlorid (1:2,000)+formic acid (1 per cent).	24	1	Lived.
23238	Do	24	1	Do.
23239	Mercuric chlorid (1:3,000)+formic acid (1 per cent).	24	1	Do.
23240	Do	24	1	Do.
23241	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Do.
23242	Do	24	1	Do.
23243	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	1	Died in 5 days. Anthrax.
23244	Do	24	1	Do.
23245	No disinfectant	(a)	1	Died in less than 48 hours. Anthrax.
23246	Do	(a)	1	Do.

EXPERIMENT 11

23251	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
23252	Do	24	1	Do.
23277	Do	24	1	Do.
23278	Do	24	1	Do.
23279	Do	24	1	Do.
23280	Do	24	1	Do.
23281	Do	24	1	Do.
23282	Do	24	1	Do.
23250	Do	24	2	Died in 5 days. Anthrax.
23248	No disinfectant	(a)	1	Died in 48 hours. Anthrax.
23249	Do	(a)	1	Do.

EXPERIMENT 12

24354	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
24355	Do	24	1	Do.
23456	Do	24	1	Do.
23457	Do	24	1	Do.
23458	Do	24	1	Do.
23459	Do	24	1	Do.
23452	Do	24	2	Do.
23453	Do	24	2	Do.
23451	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	5	Do.
23449	No disinfectant	(a)	1	Died in 48 hours. Anthrax.
23450	Do	(a)	1	Do.

EXPERIMENT 13

24999	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
25300	Do	24	1	Do.
25301	Do	24	2	Do.
25302	Do	24	2	Do.
25303	Do	24	4	Died in 3 days. Not anthrax.
25304	Do	24	4	Lived.
25315	Sodium chlorid, but no disinfectant	(a)	1	Died in 4 days. Anthrax.
25316	Do	(a)	1	Died in 5 days. Anthrax.

a Not exposed.

Since in experiment 10 mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, was shown to be efficient, while mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, was not, further tests were made with the lower dilution.

Ten pieces of hide were exposed for 24 hours to 25 c. c. (for each piece) of mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, and then soaked 24 hours in a saturated common-salt solution. The clots were then scraped off and inoculated into guinea pigs. In one instance two clots were inoculated into one animal; in all other cases only one clot was used (Table VI, experiment 11).

Another experiment (Table VI, experiment 12) was made in which six guinea pigs were inoculated with one clot each from pieces of hide disinfected with mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid; two guinea pigs were inoculated with two clots each from pieces similarly disinfected; and one guinea pig was inoculated with five clots from pieces of hide disinfected with mercuric chlorid, 1 to 2,500, plus 1 per cent of formic acid. As in the preceding experiments, each piece of hide was exposed for 24 hours to 25 c. c. of disinfectant and soaked in 25 c. c. of saturated common-salt solution for 24 hours, after which the clots were scraped off and inoculated under the skin of the guinea pigs.

The apparent discrepancy between experiments 11 and 12 in connection with results obtained by inoculation into guinea pigs of clots from two pieces of hide disinfected with mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, may be explained on the ground that the pieces used in the second experiment had been kept longer than those used in the first and had consequently lost virulence by continued drying. Even in experiment 11 it will be seen that the disinfectant exercised a marked influence on the virulence of the spores, since the guinea pig remained alive until five days after inoculation.

The results of these experiments are confirmed by the results of a further experiment (Table VI, experiment 13) performed later with test preparations of a different lot. This later lot was prepared in exactly the same way as the earlier ones; but the culture used for infecting the pieces of hide was obtained from a guinea pig dying a little more than 48 hours after inoculation, while the culture used for the pieces first prepared was obtained from a guinea pig dying within 36 hours after inoculation. The difference in the vitality of the spores is clearly seen in the length of time necessary to kill the guinea pigs inoculated from the check pieces. As will be seen by reference to Table VI, this time was about 48 hours for the first lot, while for the second it was from 4 to 5 days.

In order to ascertain the effect of mercuric chlorid and formic acid upon hides from the standpoint of the tanner, pieces of hide about 4 by 5 inches in size and weighing about 50 gm. each were disinfected by the Seymour-Jones method, using mercuric-chlorid dilutions of 1 to 4,000 and 1 to 2,500 plus 1 per cent of formic acid. The proportion of disinfectant used was 10 times the weight of the hide. These were examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry. Immediately after dehairing, these pieces of hide were observed to be very much blackened, but after the full process of tanning

this was not evident, so it appeared that the coloring matter of the tanning liquid had covered up this discoloration.

Judged solely by the results of the various experiments previously described, it might seem that the Seymour-Jones method could be accepted as suitable for the disinfection of hides, provided that mercuric chlorid in a strength of 1 to 2,500 was substituted for the recommended dilution of 1 to 5,000. However, at this stage the writer's attention was called to the work of Ševčík (14), which appeared to controvert the favorable results obtained by various workers as well as his own previous results. Ševčík concluded that it is necessary to carefully neutralize the disinfectant before attempting, either by cultural methods or animal inoculation, to ascertain whether anthrax spores have been destroyed, and that the hydrogen-sulphid solution used for a short time is not sufficient to neutralize mercuric chlorid plus formic acid. The neutralizing agent which he recommended was sodium sulphid, which neutralizes both the mercury and the acid. The time which he allowed for the neutralizing process was two hours.

Ševčík's contention that the mercuric chlorid and formic acid used in the Seymour-Jones method should be neutralized by sodium sulphid in order to determine whether disinfection has been complete seemed reasonable in view of the fact that many tanners use sodium sulphid for dehairing hides; therefore, in order to verify his conclusions, the following experiments were undertaken.

IV. EXPERIMENTS UPON PIECES OF HIDE INFECTED BY SPORES OF ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS WITH SODIUM SULPHID AS A NEUTRALIZING AGENT

Pieces of hide were exposed to 25 c. c. of disinfectant for 24 hours, treated with 25 c. c. of saturated solution of sodium chlorid for one hour and with 25 c. c. of a 1 per cent sodium-sulphid solution for two hours. They were then washed with sterile water.

In experiment 14 (Table VII) the clots were scraped off and inoculated into guinea pigs.

TABLE VII.—*Inoculation of guinea pigs with clots from infected pieces of hide*

EXPERIMENT 14

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25522	Mercuric chlorid (1:1,000) + formic acid (1 per cent).	24	Died in 3½ days. Anthrax.
25523	Do	24	Lived.
25524	Mercuric chlorid (1:2,500) + formic acid (1 per cent).	24	Do.
25525	Do	24	Died in 3½ days. Anthrax
25526	Mercuric chlorid (1:4,000) + formic acid (1 per cent).	24	Died. Mixed infection.
25527	Do	24	Died in 4 days. Anthrax.
25528	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died in 3 days. Anthrax.
25529	Do	(a)	Died. Mixed infection.

a Not exposed.

The test preparations used in experiment 14 were made as follows: Pieces of hide were cut so as to weigh about 2½ gm. A good-sized drop of guinea-pig blood was allowed to fall upon the center of each piece, and, before this clotted, a loopful of a suspension of anthrax spores was thoroughly mixed in. The suspension of spores was obtained by rubbing up in sterile water enough of the surface growth of an agar culture of *Bacillus anthracis* obtained directly from the spleen of a guinea pig (No. 25386) to give a suspension rather more dense than a 24-hour bouillon culture of *B. typhosus*. The loop employed was of No. 23 gauge platinum wire 3 mm. in diameter. The pieces of hide thus infected were dried in an electric oven at a temperature of about 45° C., in order to prevent the spores from developing into vegetative forms, which would be destroyed by the drying.

In experiment 15 (Table VIII) the test pieces of hide were prepared as follows: Pieces cut to weigh 2½ gm. were placed in a rather dense suspension of anthrax spores with hair side down. After soaking in this solution for 10 minutes they were placed in Petri dishes hair side up and allowed to dry a few minutes. Then 0.1 c. c. of the spore suspension was dropped on each piece and they were allowed to stand at room temperature for one hour. The pieces of hide were then dried in an electric oven at 43° C. for two days, the covers of the Petri dishes being tilted to one side. They were then kept at room temperature until used. After exposure to the disinfectant a considerable part of the hair with some of the underlying hide was scraped off and inoculated subcutaneously into guinea pigs, instead of inoculating blood clots as before. In other respects the technique was the same as for experiment 14.

TABLE VIII.—*Inoculation of guinea pigs with portions of hide*

EXPERIMENT 15

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25366	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Died in 3½ days. Anthrax.
25367	Do.....	24	Lived.
25368	Mercuric chlorid (1:2,500)+formic acid (1 per cent).....	24	Died in 5 days. Anthrax.
25369	Do.....	24	Do.
25370	Mercuric chlorid (1:4,000)+formic acid (1 per cent).....	24	Died in 4 days. Anthrax.
25371	Do.....	24	Died in 3½ days. Anthrax.
25372	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Do.
25373	Do.....	(a)	Died in 2 days. Mixed infection.

EXPERIMENT 16

25717	Mercuric chlorid (1:500)+formic acid (1 per cent).....		Lived.
25718	Do.....	24	Do.
25719	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Do.
25720	Do.....	24	Do.
25721	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Do.
25722	Do.....	24	Do.
25723	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 4 days. Anthrax. Do.
25724	Do.....	(a)	

a Not exposed.

TABLE VIII.—*Inoculation of guinea pigs with portions of hide*—Continued

EXPERIMENT 17

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
24598	Mercuric chlorid (1:500)+formic acid (1 per cent).....	24	Lived.
24599	Do.....	24	Died after 7 days. Anthrax.
25725	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Lived.
25726	Do.....	24	Do.
25727	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Died after 6 days. Anthrax.
25728	Do.....	24	Lived.
25729	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 3 days. Mixed infection.
25730	Do.....	(a)	Died after 5 days. Anthrax.

EXPERIMENT 18

27221	Mercuric chlorid (1:250)+formic acid (1 per cent).....	24	Died after 4 days. Anthrax.
27222	Do.....	24	Died after 6 days. Not anthrax.
27223	Mercuric chlorid (1:500)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27224	Do.....	24	Died after 6 days. Anthrax.
27225	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27226	Do.....	24	Lived.
27227	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27228	Do.....	24	Died after 6 days. Anthrax.
27231	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 5 days. Anthrax.
27232	Do.....	(a)	Died. Mixed infection.

a Not exposed.

Experiment 16 (Table VIII) was similar to the preceding, except that the pieces of hide used were dried for three instead of two days. A culture from the heart blood of guinea pig 25515 was used in making the spore suspension.

Apparently the added duration of drying had an injurious action upon the spores. It should be noted, however, that cultures from one guinea pig (No. 25386) were used in preparing material in experiments 14 and 15, while the test preparations used in experiment 16 were infected by a culture derived from a different animal.

The available cultures from the same source as those used in preparing material for experiments 14 and 15 were now 1 month old. In experiment 17 (Table VIII) one of these was used in infecting pieces of hide in the following way: Pieces of hide of 2½ gm. weight were soaked in a suspension of anthrax spores for 10 minutes; then one-tenth c. c. of suspension was dropped on each, and the pieces of hide were dried in an electric oven at 43° C. for 24 hours and then kept at room temperature for 24 hours before use. The covers of the Petri dishes containing the pieces of hide were kept raised during all of this time.

The results of this experiment seem to indicate that cultures derived from one animal (guinea pig 25386) yielded spores of very great resisting power as compared with cultures from another animal (guinea pig 25515). The irregularities which will be noted in experiment 17 are probably due to the age of the culture used.

A further series of experiments having given unsatisfactory results, it was deemed advisable to undertake comparative tests of infected pieces of hide prepared by several different methods.

Further experiments were thereupon made to compare the infectivity of pieces of hide dried (1) in an electric oven at 44° C. for 40 hours; (2) in an incubator at 37° C. for 24 hours (spores in blood clots); and (3) in a desiccator over sulphuric acid at a temperature of about 10° C., the desiccator being exhausted of air down to a pressure of about 6 cm. of mercury, time of drying, 48 hours.

Of the above only those pieces dried at a low temperature proved infectious, the guinea pig inoculated dying after one week. As a guinea pig inoculated by pure culture also remained alive for a week, it seemed that the process of drying at 10° C. in a vacuum over sulphuric acid had not appreciably diminished the virulence of the spores. This process was therefore used in the preparation of all further test pieces of hide.

Previous experiments had shown a difference between the two strains of guinea pigs which had been used in these experiments, one strain being much more susceptible to infection by anthrax than the other. The comparatively low virulence of the pure culture mentioned above seemed to be due to passage through the less resistant strain of guinea pigs. Beginning, therefore, with a culture which had not been so treated, successive inoculations were made with the more resistant strain of guinea pigs until cultures of satisfactory virulence and vitality were obtained.

V. EXPERIMENTS UPON INFECTED PIECES OF HIDE DRIED AT 10° C.

A lot of pieces of hide were prepared as follows: Pieces of 2½ gm. in weight were washed and dried. These were infected by a suspension made from a 7-day agar culture, in the following manner: Pieces were placed in the suspension, hair side down, and allowed to soak for 10 minutes, and then 0.2 c. c. of the suspension was dropped on each. These pieces were left in Petri dishes in the ice box for half an hour with covers of dishes on. At the end of that time the dishes were placed in a desiccator over sulphuric acid and the covers raised. The desiccator was then exhausted of air and put into the ice box, where it remained 48 hours at a temperature of 10° C. The pieces of hide were then removed and kept at room temperature until used. A guinea pig inoculated with the pure culture used for infecting these pieces of hide died in four days. Using the pieces of hide prepared as described, the following experiments were performed:

In experiment 18, pieces of hide were exposed to the disinfectant for 24 hours, followed by a saturated salt solution for 1 hour. They were then treated with a 1 per cent sodium-sulphid solution for 2 hours and washed with sterile water. Material was then scraped from the surface of each and inoculated into a guinea pig. The results are given in Table VIII, experiment 18.

In this experiment, as in those of similar character preceding it, neutralization of the disinfectant by sodium sulphid was done within a comparatively few hours after the process of disinfection was complete. In view of the strong dilution (1 to 250) found to be inefficient under these circumstances, no further attempt was made to find a dilution strong enough to disinfect, with neutralization afterward. Instead of this, an attempt was now made to determine how long spores remained viable after treatment of the pieces of hide by much weaker dilutions of mercuric chlorid plus formic acid. This seemed worth while because the Seymour-Jones method was originally proposed to be employed at foreign ports, and in a voyage of ordinary length a considerable time would thus elapse between the time of disinfection and time of arrival at destination.

In experiment 19 (Table IX) a number of pieces of hide were exposed for 24 hours to mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, treated with saturated common salt for 1 hour, and then laid aside and at intervals treated with sodium sulphid and inoculated into guinea pigs. In each case they were treated with 1 per cent of sodium sulphid for 2 hours and washed with sterile distilled water. Material was then scraped from each piece and inoculated subcutaneously into a guinea pig.

TABLE IX.—*Inoculation of guinea pigs with infected portions of hide*

EXPERIMENT 19 [a]

Guinea pig No.	Disinfectant (25 c. c.) and dilution	Time of exposure	Time before treatment with sodium sulphid.	Result of inoculation.
		Hours.	*Days.*	
27229	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
27229	Do	24	1	Do.
27257	Do	24	2	Died after 6 days. Anthrax.
27258	Do	24	2	Died. Pneumonia.
27259	Do	24	3	Died after 6½ days. Anthrax.
27260	Do	24	3	Lived.
27261	Do	24	4	Died after 10 days. Anthrax.
27262	Do	24	4	Died. Pneumonia.

EXPERIMENT 20 [b]

		Hours.	*Days.*	
27521	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
27522	Do	24	1	Do.
27525	Do	24	3	Died after 6 days. Anthrax.
27526	Do	24	3	Lived.
27531	Do	24	6	Died after 4 days. Anthrax.
27532	Do	24	6	Died after 5 days. Anthrax.
			Weeks.	
28004	Do	24	2	Lived.
28005	Do	24	2	Do.

[a] Control guinea pig died of anthrax in 5 days.
[b] Control guinea pig died of anthrax in 7 days

TABLE IX.—*Inoculation of guinea pigs with infected portions of hide*—Continued

EXPERIMENT 21 [a]

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Time before treatment with sodium sulphid.	Result of inoculation.
		Hours.	*Days.*	
28006	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
28007	Do	24	1	Do.
28010	Do	24	4	Died after 9 days. Anthrax.
28011	Do	24	4	Died after 3 days. Anthrax.
28056	Do	24	9	Died after 7 days. Anthrax.
28057	Do	24	9	Died after 6 days. Anthrax.
			Weeks.	
28072	Do	24	2	Do.
28073	Do	24	2	Died after 8 days. Anthrax.

EXPERIMENT 22 [b]

		Hours.	*Days.*	
28012	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Died after 9 days. Anthrax.
28013	Do	24	1	Lived.
28008	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	24	1	Died after 13 days. Anthrax.
28009	Do	24	1	Lived.
28052	Do	24	2	Do.
28053	Do	24	2	Do.
28054	Do	24	4	Died after 6 days. Anthrax.
28055	Do	24	4	Lived.
28058	Do	24	6	Do.
28059	Do	24	6	Do.

[a] Control guinea pig died of anthrax in 4 days.
[b] Control guinea pig died of mixed infection.

The irregular results noted above might be due to variation in the extent of infection of the various pieces of hide.

In another experiment with similar technique, except that the pieces of hide were infected at a different time and by a different culture, the results were as given in Table IX, experiment 20.

In experiment 21 (Table IX), also, the procedure was the same as in experiment 19, except that the test pieces of hide were infected by a different culture.

Experiment 22 (Table IX) was similar to the preceding experiments, except in the use of a stronger dilution of the disinfectant.

In connection with experiments 20, 21, and 22 part of the material scraped from the pieces of hide was plated out to determine whether sterilization had been accomplished. Growth of some kind was obtained in every instance, although *Bacillus anthracis* was isolated in only about one-third of the cases. In one instance *B. anthracis* was recovered from material which failed to cause anthrax when inoculated into guinea pigs, but on the other hand, one guinea pig died from anthrax after inoculation with material which failed to yield *B. anthracis* by the plate method.

HYDROCHLORIC ACID AND SODIUM CHLORID

In view of the apparent inefficiency of the Seymour-Jones method and the favorable results reported by various workers using the Schattenfroh method, experiments were now undertaken to determine the germicidal power of hydrochloric acid and sodium chlorid against anthrax spores, both as "naked" spores and as contained on and in infected pieces of hide. The Schattenfroh method (12) as described by Prof. Schattenfroh consists of immersion of hides in solutions of hydrochloric acid and common salt, the proportions recommended varying according to temperature. The proportions recommended for use at room temperature are 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid, with the time of exposure 48 hours. At higher temperatures less of the acid is needed and the time of exposure is shortened, but inasmuch as special apparatus would be needed to maintain these higher temperatures it seemed that disinfection at these higher temperatures could be disregarded as being of little practical significance.

The experiments here described were therefore carried on at room temperature. In all cases dilutions were calculated upon the percentage of absolute hydrochloric acid, not upon the percentage of "concentrated hydrochloric acid." In accordance with Schattenfroh's recommendations, a sodium-carbonate solution was used after exposure to the disinfectant, in order to neutralize the hydrochloric acid.

1. EXPERIMENTS BY THE ROD METHOD, USING SPORES OF BACILLUS ANTHRACIS

A series of experiments was first made by the rod method, using various proportions of hydrochloric acid and sodium chlorid. The time of exposure in each case was 24 hours, and rods were washed with a 2 per cent solution of sodium carbonate to neutralize the hydrochloric acid. Experiment 23 (Table X) was made without the addition of organic matter; experiment 24 (Table X) was made with the addition of 1 c. c. of defibrinated blood to 9 c. c. of disinfectant in each tube. The results are given in Table X, together with the results of an experiment upon mercuric chlorid, alone and with acetic acid and formic acid, which was made at the same time, and with rods infected by the same spore suspension. This suspension was rather heavier than usual. In experiment 24 the hydrochloric-acid rods were washed in a 20 c. c. sodium-carbonate solution for one minute, and the mercuric chlorid rods in a 20 c. c. saturated hydrogen sulphid for one minute.

TABLE X.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid and mercuric chlorid, with and without formic acid, and with acetic acid, by the rod method, without addition of organic matter*[a]

EXPERIMENT 23

Rod No.	Disinfectant (10 c. c.) and dilution	Time of exposure	Result
		Hours.	
1	Hydrochloric acid (1 per cent)+sodium chlorid (5 per cent)	24	No growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (5 per cent)	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (5 per cent)	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (5 per cent)	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (5 per cent)	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent)	24	Do.
9	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent)	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent)	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent)	24	Do.
12	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent)	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent)	24	Do.
14	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent)	24	Do.
15	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent)	24	Do.
16	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent)	24	Do.
17	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent)	24	Do.
18	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent)	24	Do.
19	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent)	24	Do.
20	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent)	24	No Growth.
21	Control rod	(b)	

EXPERIMENT 24[c]

1	Hydrochloric acid (1 per cent)+sodium chlorid (5 per cent)	24	Growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (5 per cent)	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (5 per cent)	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (5 per cent)	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (5 per cent)	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent)	24	No growth.
9	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent)	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent)	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent)	24	Growth.
12	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent)	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent)	24	No growth.
14	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent)	24	Do.
15	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent)	24	Do.
16	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent)	24	Do.
17	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent)	24	Growth.
18	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent)	24	No growth.
19	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent)	24	Growth.
20	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent)	24	No growth.
21	Mercuric chlorid (1:1,000) alone	24	Growth.
22	Mercuric chlorid (1:1,000)+acetic acid (1 per cent)	24	Do.
23	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)	24	Do.
24	Mercuric chlorid (1:3,000)+acetic acid (1 per cent)	24	Do.
25	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	Do.
26	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Do.
27	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
28	Control rod	(b)	Do.

EXPERIMENT 25[c]

1	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	No growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent)	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent)	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent)	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent)	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent)	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent)	24	Do.
9	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent)	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent)	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent)	24	Do.
12	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent)	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent)	24	Do.
14	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent)	24	Do.

[a] Percentage of hydrochloric acid means percentage of absolute hydrochloric acid.
[b] Not exposed.
[c] The quantity of disinfectant used (10 c. c.) included 1 c. c. of defibrinated blood.

TABLE X.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid and mercuric chlorid, with and without formic acid, and with acetic acid, by the rod method, without addition of organic matter*—Continued

EXPERIMENT 25—continued

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result
		Hours.	
15	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent).	24	No growth.
16	Mercuric chlorid (1:500) alone	24	Growth.
17	Mercuric chlorid (1:1,000) alone	24	Do.
18	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)	24	Do.
19	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)	24	Do.
20	Mercuric chlorid (1:3,000)+acetic acid (1 per cent)	24	Do.
21	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	No growth.
22	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Do.
23	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Growth.
24	Control rod	(a)	Do.

[a] Not exposed.

A similar experiment (Table X, experiment 25) was made with rods infected by a spore suspension of about the same density as a 24-hour bouillon culture of *Bacillus typhosus*.

In experiments 26 and 27 (Table XI) are shown a comparison of hydrochloric acid and common salt with several other disinfectants, all with 24-hour exposure. Three rods were used with each dilution, showing the result, respectively, when no defibrinated blood was added, with ½ c. c. of blood added to each tube and with 1 c. c. of blood added to each tube. The hydrochloric-acid rods were washed with a 2 per cent sodium-carbonate solution, the mercuric-chlorid rods with a saturated hydrogen-sulphid solution, and the formalin and carbolic-acid rods with distilled water.

TABLE XI.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid, formalin, phenol, and mercuric chlorid, with and without formic acid, by the rod method*

EXPERIMENT 26

Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.		
		No blood added.	½ c. c. blood added.	1 c. c. blood added.
	Hours.			
Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent).	24	No growth.	No growth.	No growth.
Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	24	.....do.....	.....do.....	Do.
Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent).	24	.....do.....	.....do.....	Do.
Mercuric chlorid (1:1,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Growth.
Mercuric chlorid (1:2,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Do.
Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	.....do.....	Growth.....	Do.
Mercuric chlorid (1:8,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Do.
Mercuric chlorid (1:16,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Do.
Formalin (1:50)	24	.....do.....	No growth.	Do.
Formalin (1:100)	24	.....do.....	Growth.....	Do.
Formalin (1:250)	24	Growth.....	.....do.....	Do.
Formalin (1:1,000)	24	.....do.....	.....do.....	Do.
Phenol (5 per cent)	24	.....do.....	.....do.....	Do.

TABLE XI.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid, formalin, phenol, and mercuric chlorid, with and without formic acid, by rod method*—Continued

EXPERIMENT 27

Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.	
		½ c. c. blood added.	1 c. c. blood added.
	Hours.		
Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	No growth	No growth.
Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	do	Do.
Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	do	Do.
Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	do	Growth.
Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Growth	Do.
Mercuric chlorid (1:6,000)+formic acid (1 per cent)	24	do	Do.
Mercuric chlorid (1:8,000)+formic acid (1 per cent)	24	do	Do.
Formalin (1:50)	24	No growth	Do.
Formalin (1:100)	24	Growth	Do.
Formalin (1:200)	24	do	Do.

The technique of experiment 27 was the same as that of No. 26. In this case two rods were used with each dilution, showing results with ½ c. c. and 1 c. c. of defibrinated blood.

II. EXPERIMENTS UPON PIECES OF HIDE INFECTED WITH SPORES OF BACILLUS ANTHRACIS

In experiment 28 (Table XII) a 2 per cent hydrochloric-acid solution plus 10 per cent of sodium chlorid was used with a 48-hour exposure, 25 c. c. of the disinfectant being used for each piece of hide. After exposure the pieces of hide were soaked for 15 minutes in a 3 per cent solution of sodium carbonate (25 c. c. for each). The pieces of hide used were prepared by the method given by Ponder (9, 10) and were part of the same lot as the pieces used in experiment 14. After disinfection the clots were scraped off and inoculated subcutaneously into guinea pigs.

TABLE XII.—*Inoculation of guinea pigs with clots scraped from pieces of hide*

EXPERIMENT 28

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots used.	Result of inoculation.
		Hours.		
25556	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	48	2	Lived.
25557	Do			
25558	Do	48	2	Do.
25559	Do	48	1	Do.
25560	Do	48	1	Do.
25561	Do	48	1	Do.
25562	Do	48	1	Do.
25563	Do	48	1	Do.
25554	No disinfectant	48	1	Do.
25555	Do	(a)		Died. Anthrax.
		(a)		Do.

a Not exposed.

Experiment 29 (Table XIII) was made as follows: Pieces of hide were prepared by soaking in spore suspension and then drying in an electric oven. Details given in connection with experiment 15 will apply to this experiment. The technique otherwise was the same as that of experiment 28. Material was scraped from the surface of each piece and inoculated subcutaneously into guinea pigs.

TABLE XIII.—*Inoculation of guinea pigs with material scraped from pieces of hide*

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
	EXPERIMENT 29		
		Hours.	
25710	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
25711	Do	48	Do.
25712	Do	48	Do.
25713	Do	48	Do.
25714	Do	48	Do.
25715	No disinfectant	(a)	Died in 3½ days. Anthrax.
25716	Do	(a)	Died in 6 days. Anthrax.
	EXPERIMENT 30		
25733	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
25734	Do	48	Do.
25735	Do	48	Do.
25736	Do	48	Do.
25737	Do	48	Do.
25738	Do	48	Do.
25739	Do	48	Do.
25740	Do	48	Do.
25741	Do	48	Do.
24742	Do	48	Do.
25729	No disinfectant	(a)	Died. Mixed infection.
25730	Do	(a)	Died after 5 days. Anthrax.
	EXPERIMENT 31		
27593	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
27594	Do	48	Do.
27595	Do	48	Do.
27596	Do	48	Do.
27597	Do	48	Do.
27598	Do	48	Do.
27599	Do	48	Do.
28000	Do	48	Do.
28001	No disinfectant	(a)	Died after 4 days. Anthrax.
28002	Do	(a)	Died after 3 days. Anthrax.

[a] Not exposed.

Experiment 30 (Table XIII) was made upon pieces of hide prepared in the same way but infected with a different culture.

In experiment 31 (Table XIII) the pieces of hide were prepared by soaking in spore suspension and drying them over sulphuric acid in a vacuum at 10° C. for 48 hours. As before, each piece of hide after disinfection was immersed for 15 minutes in 25 c. c. of a 3 per cent sodium-carbonate solution.

In connection with experiment 31 an attempt was made to determine the efficiency of disinfection by plating out material from the piece of

hide. The plates showed no growth even after three days' incubation; hence, it seemed that the hydrochloric acid and sodium chlorid had destroyed the anthrax spores and all other organisms as well.

Experiments 32 and 33 (Table XIV) show comparative tests of the Seymour-Jones and Schattenfroh methods upon pieces of hide of the same lot. These were prepared by the method described under experiment 31. The greatest possible care was taken to neutralize the disinfectant, so far as the Schattenfroh method was concerned. Sodium sulphid was used both for Seymour-Jones and Schattenfroh pieces, because it seemed possible that the depilatory action of the sodium sulphid might bring up undisinfected spores from the depths of the hair follicles. A number of pieces of disinfected hide were kept several days and then treated with the neutralizing agent.

TABLE XIV.—*Comparison of Seymour-Jones and Schattenfroh methods of disinfecting hides*

EXPERIMENT 32

Guinea pig No.	Disinfectant (25 c.c.) and dilution.	Neutralizing solution and time required.	Time of exposure.	Result of inoculation.
			Hours.	
28556	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	Sodium carbonate (2 per cent), ½ hour.	48	Lived.
28557	Do	do	48	Do.
28558	Do	Potassium hydroxid (0.5 per cent), 2 hours.	48	Do.
28559	Do	do	48	Do.
28560	Do	Sodium sulphid (1 per cent), 2 hours.	48	Do.
28561	Do	do	48	Do.
28562	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	do	24	Do.
28563	Do	do	24	Do.
	Neutralization 4 days later.			
28588	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	Sodium sulphid (1 per cent), 2 hours.	24	Died. Anthrax.
28589	Do	do	24	Do.
28590	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	do	48	Lived.
28591	Do	do	48	Do.

EXPERIMENT 33

28735	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	Sodium carbonate (2 per cent), ½ hour.	48	Lived.
28736	Do	do	48	Do.
28737	Do	Potassium hydroxid (0.5 per cent), 2 hours.	48	Do.
28738	Do	do	48	Do.
28739	Do	Sodium sulphid (1 per cent), 2 hours.	48	Do.
28740	Do	do	48	Do.
28729	Mercuric chlorid (1: 2,500)+formic acid (1 per cent).	do	24	Do.
28730	Do	do	24	Died. Anthrax.
	Neutralization 4 days later.			
28773	Mercuric chlorid (1: 2,500)+formic acid (1 per cent).	Sodium sulphid (1 per cent), 2 hours.	24	Died. Anthrax.
28774	Do	do	24	Lived.
28771	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	do	48	Do.
28772	Do	do	48	Do.

As a part of experiment 32, plates were made from the material scraped off the pieces of hide. In every instance the plates made from material treated by 2 per cent of hydrochloric acid and 10 per cent of sodium chlorid were sterile. On the other hand, growth was observed on all the plates from material exposed to mercuric chlorid and formic acid.

In this experiment, as in several of the last few experiments described in the previous discussion of the Seymour-Jones method, it will be noted that material from pieces of hide exposed to mercuric chlorid and formic acid and treated shortly after completion of the disinfection with sodium sulphid failed to kill guinea pigs into which it was inoculated. On the other hand, material from pieces of hide allowed to stand for several days before using sodium sulphid caused guinea pigs to die from anthrax. It was noted that the depilatory action of the sodium sulphid was far more complete in the case of the pieces of hide which had been kept for several days after disinfection before treatment with the sulphid. The results of plating, as before mentioned, showed that disinfection was not complete; therefore it seems probable that the more extensive depilatory action of the sodium sulphid upon pieces which had stood for some time brought up from the depths of the hair follicles spores which had been practically untouched by the disinfectant. It also seems possible that there had been some development and multiplication of these uninjured organisms during the period of waiting.

It should be noted that in the preparation of the pieces of hide used in all the above-mentioned experiments particular care was taken to secure penetration of the spores into the pieces of hide. In order to accomplish this, the pieces of hide after being infected by spore suspensions were placed in closed Petri dishes and kept in the ice box for four or five hours before the drying process was begun.

As will be seen by reference to Table XIII, the Schattenfroh method was entirely successful in every instance, and the results of plating showed that actual sterilization was accomplished.

Experiment 33 (Table XIV) was exactly similar to the preceding experiment except that the pieces of hide used were infected by spores derived from a different culture. The method of preparation was the same as that described under experiment 31.

In this experiment, as in the preceding one, the efficiency of the disinfectants was tested by plating out material from the pieces of hide. The results obtained varied from the results of experiment 32 in that a few colonies were found on two plates from material treated with hydrochloric acid and salt, while all other plates from similar material were sterile. One plate from material neutralized by 0.5 per cent of potassium hydrate showed two colonies, while the other, from material neutralized by sodium carbonate, showed one colony. In none of the three was *Bacillus anthracis* the organism present. Therefore, although hydro-

chloric acid and salt did not accomplish actual sterilization in every instance, it did destroy anthrax spores in every instance.

Several pieces of hide about 50 gm. weight each were exposed to 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid for 48 hours and thoroughly washed with 3 per cent sodium-carbonate solution. They were then examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry, along with pieces of hide which had been treated by other disinfectants. This work was in charge of Mr. F. P. Veitch, and the result is shown in his memorandum on page 91.

OTHER DISINFECTANTS

Bacteriological tests were made with formalin and phenol, and pieces of hide treated by these disinfectants were examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry. Without going into details it may be stated that, so far as could be determined by the limited number of tests, 2½ per cent of formalin is efficient bacteriologically both against anthrax spores and against other organisms, while 5 per cent of phenol is fairly efficient against non-spore-bearing organisms, but is practically useless against anthrax spores. It should be noted also that pieces of hide disinfected by formalin in 2½ per cent solution were so seriously affected by the disinfectant that it was almost impossible to tan them, while pieces treated with carbolic acid were uninjured.

A few tests were made of the germicidal efficiency of mercuric-chlorid solutions saturated with sodium chlorid. It was found that this combination is, if anything, not as efficient as mercuric chlorid alone. This is presumably due to interference of the salt with the ionization of the mercuric chlorid, as the work of Krönig and Paul (6) quite clearly indicates.

During the course of the investigations herein recorded, the writer noted considerable variations in the vitality and virulence of anthrax spores from different sources. It was also noted that the processes employed in infecting and drying test preparations exercised a variable influence upon the vitality of the spores. In view of these variations, it was found to be necessary to repeat the tests many times, and in order to test the various methods as thoroughly as possible, every effort was made to maintain at the highest possible point the vitality and virulence of the spores used in test preparations and to make sure of the presence of a considerable number of such spores upon each test preparation.

It seems likely that anthrax spores occurring upon naturally infected hides might in many cases be present in much smaller numbers and possess far less vitality and virulence than those used in the experiments. However, in view of the results obtained by Ševčík (14) and

others working with naturally infected hides, it is evident that the spores upon such hides frequently possess very high vitality and virulence. Therefore it seems that the only safe rule to follow is to use only such disinfectants and such methods of disinfection as have been found efficient against spores of maximum vitality and virulence.

SUMMARY AND CONCLUSIONS

(1) THE SEYMOUR-JONES METHOD.—The strength of disinfectant originally recommended by Seymour-Jones (mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid) was not found to be efficient, even without neutralization of the disinfectant. A stronger dilution, 1 to 2,500, plus 1 per cent of formic acid, was found to be efficient where no neutralization was attempted. The latter strength was not sufficient, however, to prevent fatal infection of guinea pigs by disinfected material when the disinfectant was neutralized by a 1 per cent sodium-sulphid solution three or four days after the completion of the process of disinfection. No infection was caused by the inoculation of material which had been kept a week or more after disinfection. It seems, therefore, that the Seymour-Jones method might be employed with dilutions of mercuric chlorid, 1 to 2,500, plus 1 per cent of formic acid, provided the treated hides are not to be subjected within a week or two to the action of any substance which will neutralize the disinfectant. This would be the case, for instance, if hides were disinfected at foreign ports before shipment to this country.

(2) THE SCHATTENFROH METHOD.—Hydrochloric acid and sodium chlorid in the proportions of 2 per cent of the acid and 10 per cent of the salt and with 48 hours' exposure have proved efficient in every instance. Consequently from the bacteriological standpoint the Schattenfroh method seems to be entirely satisfactory. This conclusion is supported not only by this work but by the exhaustive researches of Gegenbauer and Reichel (3) and Hilgermann and Marmann (4). The recently published work of Ševčík (15) is not so favorable to the Schattenfroh method as that of the investigators previously mentioned. He finds that complete disinfection can be accomplished when the hides worked with are thin. But when the hides are thick and heavily infected, he was able, after very thorough neutralization, to extract from pieces of the treated hides anthrax spores which were virulent for mice, and in some instances for guinea pigs, even after exposure to a solution of 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid for 7 days.

Although in view of the above-mentioned results the Schattenfroh method can not be regarded as perfect, it nevertheless seems to be far superior to other methods and well worth a trial as a standard method for the disinfection of hides.

(3) EFFECT OF DISINFECTION UPON HIDES AS REGARDS TANNING.—Mr. F. P. Veitch, Chemist in Charge of the Leather and Paper Laboratory

of the Bureau of Chemistry, has been kind enough to furnish the following memorandum in regard to the tanning of small pieces of normal hide treated by the Seymour-Jones and Schattenfroh processes of disinfection.

No marked differences in color were noted among the various pieces of tanned leather. Slight differences, due to difference in thickness, were noted in pliability, but these did not appear to be connected with the disinfecting treatment. No marked difference could be detected in the appearance of the grain of the leather. All the pieces cracked when severely bent, owing probably to excessive tannin in the grain of the leathers. The treated leathers did not display more pronounced cracking than those which were not treated. Microscopical examination of the hide fibers after deliming and of the leather fibers after tanning shows no marked differences among the several pieces of hide.

The results in general seem to indicate that the several treatments have not injured the hides. The evidence, however, is not sufficient to permit of definite conclusions being drawn at this time. More extended work in commercial tannery, using whole hides, has been planned to determine definitely whether any of the disinfectants result in the production of inferior leather. Since tanning is a slow process, it will require from nine months to a year to secure these data.

Mr. Veitch also states that all the leathers gave reactions for chlorids, but that the leathers treated with disinfectants apparently contained larger amounts of chlorids than the other leathers.

It seems, then, so far as the evidence at hand permits any conclusion at all, that neither the Seymour-Jones method nor the Schattenfroh method exerts any injurious effect upon hides or leather.

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OBSERVATIONS ON RHIZINA INFLATA

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Considerable doubt exists regarding the parasitism of *Rhizina inflata* (Schäff.) Sacc. (*R. undulata* Fr.). This peculiar fungus (Pl. VIII, figs. 1, 2, and 3) occurs quite abundantly on the ground in the forest-fire areas of the Northwest. Usually found as a saprophyte on the burned forest soil, it attracted little attention until the close proximity of the fruiting bodies to dead coniferous seedlings was noted to be of frequent occurrence. A close examination of the roots of the dead seedlings showed the mass of white mycelium clinging to and ramifying in the cortical tissues of the root to be in connection with the near-by fruiting structures of *Rhizina inflata*. In some cases the sporophores of this fungus surrounded the stem of the seedling.

The observations on the parasitism of this fungus are not extensive. The disease "la maladie du rond" of *Pinus sylvestris* and *P. maritima*, according to the investigations of Prillieux and De la Boulaye (1880),[1] is accredited to this fungus. Hartig (1891, 1892, 1894, p. 123–129) afterwards in more thorough investigations substantiated the observations of the former investigators and showed *Rhizina inflata* to be capable of living as a true parasite, causing the death of 4-year-old seedlings of *Abies pectinata*, *Pinus strobus*, *Larix europaea*, *Picea sitkaensis*, *Tsuga mertensiana*, *Pseudotsuga douglasii*, and *Castanea vesca*. Von Tubeuf (1897, p. 273) also reports the fungus as a parasite in the forest-tree nurseries of Germany and in the natural forests of *Pinus pinaster* in France.

Early in the spring of 1912 at a certain point along an old logging road in the Kaniksu National Forest, Idaho, where the brush had been burned, young 3- to 5-year-old seedlings of *Tsuga heterophylla*, *Larix occidentalis*, and *Pinus monticola* were observed to be dying in small isolated patches. The roots of the seedlings on being pulled up were closely matted together by a white mycelium, causing a quantity of earth to adhere to them. Since fungous fruiting bodies were not in evidence on any part of the diseased plants or on the ground around, the death of the seedlings was attributed to *Armillaria mellea* (Vahl) Quél., which is very abundant in this region and is frequently the cause of the death of very young growth. The mycelium had penetrated all parts of the cortical and bast tissues of the roots, causing them to become saturated with resin, a condition quite similar to that produced by *A. mellea*. The diseased areas were

[1] Citations to literature in parentheses refer to "Literature cited," p. 92.

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from 2 to 4 feet in diameter and were irregularly circular in shape, as if the causal agent had started from the center.

Later in the season, near the borders of these areas and at the base of the stems of the dead seedlings, deep-brown, effused, undulating, fruiting structures appeared, which were at once recognized as those of *Rhizina inflata* (Pl. VIII, fig. 2). As to the connection of these fruiting structures with the mycelium beneath them in the forest mold and with that of the roots of the diseased seedlings, there seemed little room for doubt. It did not seem probable that the base of the diseased plant would be completely inclosed by the fruiting structure, with its peculiar rootlike fibrils (Pl. VIII, fig. 3) mingling with the mass of mycelium about the diseased roots, without having some connection with it. Such a seedling with fruiting body attached was carefully removed from the soil and placed in a dish of water, in order to allow the attached earth to fall gradually away. It was found that the numerous rhizoids or strands of mycelium by which the fruiting structures are attached to the substratum were continuous with the mycelium surrounding the diseased roots. These roots were microscopically examined and showed that the internal mycelial system ramifying in the cortical parenchyma and in the sieve tubes of the bast was a continuation of the mycelium which connected up the rhizoid strands of the fruit body.

By shaking in boiled water a quantity of soil which had been burned over the previous year and which showed no signs of fungous growth, a solution was prepared to which a large quantity of spores of *Rhizina inflata* was added. This solution was thoroughly sprayed about the base of several healthy 3- to 4-year-old white-pine seedlings (*Pinus monticola*) growing on burned ground in another part of the forest. The sprayed seedlings appeared slightly reduced in vigor in the fall of 1912 and by July of 1913 they were dead. The roots of each were infected by the same clinging mass of mycelium previously described. The stems and leaves were free from any other diseases. It is believed that this result, although not obtained under control conditions, furnishes some experimental proof of the parasitism of *Rhizina inflata* as it occurs in the Northwest.

Underwood (1896) reports the distribution of the species as follows: Connecticut (Thaxter), New York (Peck), Rhode Island (Bennett), Pennsylvania (Schweinitz), Wisconsin (Bundy), North Carolina and South Carolina (Curtis). The range of *Rhizina inflata* is further extended by the writer, who has collected it at the following stations: Priest River, Idaho, in Kaniksu National Forest on *Pinus monticola*, *Tsuga heterophylla*, and *Larix occidentalis;* Coeur d'Alene National Forest, Idaho, on *Pinus monticola* and *Abies grandis;* Thompson Falls, Mont., in Cabinet National Forest, on *Pinus contorta;* Missoula National Forest, Mont., on *Pinus ponderosa;* Lolo National Forest, Mont., on *Pinus monticola;* Ely, Minn., in Superior National Forest, on *Pinus divaricata;* and Salmon Arm, British Columbia, on *Pseudotsuga taxifolia*.

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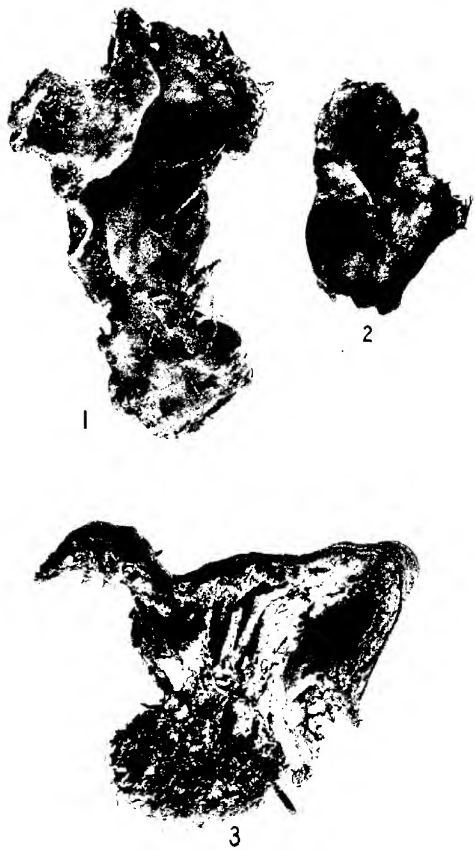
PLATE VIII

Fig. 1.—Mature fruiting structure of *Rhizina inflata*, showing the undulating upper surface.

Fig. 2.—Immature fruiting structure of *Rhizina inflata*.

Fig. 3.—Fruiting structure of *Rhizina inflata*, showing the peculiar mycelial strands or fibrils by which the fruiting body is attached to the substratum.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PSEUDOMONAS CITRI, THE CAUSE OF CITRUS CANKER

[A PRELIMINARY REPORT]

By CLARA H. HASSE,

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During the summer of 1914 reports of the rapid spread of Citrus canker and the severe injury caused by this new Citrus disease were received by the Bureau of Plant Industry from orange and grapefruit growers in Florida, Texas, and Mississippi. It soon became evident that this disease was one of unusual virulence, which made the investigation of its cause a matter of urgent importance. From the reports of various investigators it appears that Citrus canker was known and recognized as a new disease before any specimens were received by this Bureau.[1] The first specimens received by the Bureau consisted of fruits, leaves, and twigs of grapefruit and showed cankers in every stage of development, from the youngest infections, which were scarcely more than a millimeter in diameter, to the large corky forms, as much as 5 mm. in diameter. A careful microscopic study was made of some of the youngest cankers, and the presence of bacteria was immediately detected. Bacteria were found in fresh sections and have been demonstrated in a large number of stained sections, as represented in the accompanying illustration (Pl. IX, fig. 1).

Numerous plate cultures were made from fresh specimens of cankers received at different times, and an organism was isolated which has been proved to be pathogenic to grapefruit seedlings.

Due attention has been given to all the rules governing bacteriological technique, and every precaution has been observed in making the inoculations. The inoculations were made on young, healthy, vigorously growing grapefruit seedlings, which were kept in the laboratory because the highly infectious nature of the disease made it impossible to carry on the experiments in the Department greenhouses. Pure cultures of the organism were mixed with sterile distilled water, and the suspension thus obtained was placed upon the upper and the under leaf surfaces by means of a sterile pipette in such a manner that the leaves were, for a short time at least, covered with a film of the inoculating fluid. The main stem and branches were treated in the same way. In some cases the leaves and stems were punctured with a sterile needle, but this is not

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necessary, as infections may be obtained without this procedure. As soon as the plants were inoculated they were placed under bell jars and kept at a temperature of about 86° F. Under these conditions the organism takes a vigorous hold on its host, and in three or four days evidences of infection can be noted. At the end of a week definite, well-defined cankers which penetrate the tissue of the leaf have been formed. Owing to the stimulating influence which the organism has upon the infected leaf tissue, there is a rapid development of cells, and the tension resulting from the abnormal growth quickly ruptures the epidermis and exposes the soft, spongy, underlying canker tissue, which is distinctly visible on both sides of the leaf. The cankers produced by artificial inoculation present a characteristic appearance and closely resemble natural cankers in macroscopic as well as in microscopic features. They penetrate the tissue of the leaf and are more or less raised on both the upper and the lower surface. The outline is circular, and there is a sharp, distinct demarkation between the canker and the surrounding normal leaf tissue. Young cankers have a soft, spongy structure and at first show a light-green color, which later turns red-brown. The cells in the canker tissue become suberized and produce a corky growth, which is a symptom of the disease. This open, spongy type of canker is the result of rapid growth due to favorable conditions of temperature and moisture.

The identity of natural and artificial cankers is shown in Plate X. Sections of cankers about 2 weeks old show the pathological and histological features observed in young natural infections. (See Pl. IX, fig. 1.) The cells are found to be filled with short rod bacteria, and the stimulus exerted by the organism on the infected tissue is distinctly visible. The natural differentiation of palisade and parenchyma tissue has been obliterated, and all the cells exhibit more or less enlargement and distortion, which is due to the activity of the invading organism. As a result the diseased tissue of the canker is raised above the normal leaf surface. In later stages in the development of the canker some of the cells disintegrate, and lesions are formed. The organism appears to act more vigorously on the cell contents than on the cell walls, and in due time the cell contents are exhausted. The cell walls which remain become suberized and constitute the corky cankerous growth which is a characteristic symptom of this disease. Numerous cankers obtained from pure-culture inoculations upon grapefruit seedlings are shown in Plate IX, figures 3, 4, 5, 6.

While the canker is still soft and young, the organism is in a very active condition and can be isolated very readily. Upon teasing out a small piece of canker tissue in a drop of sterile water, motile bacteria in great numbers ooze out and give the water a milky, turbid appearance. The motility of the organism can be most satisfactorily observed by means of dark-field illumination. The organism was reisolated from

these cankers by plating out on beef agar and was found to be identical with the original organism. Inoculations on grapefruit plants with the organism obtained from this reisolation produced characteristic cankers.

The open surface of the canker and the spongy character of its structure afford an excellent lodging place for spores of all sorts, and it is not surprising to find fungi, some of which may perhaps play a minor part in the later stages of the disease. A number of fungi have been isolated from old Citrus cankers, and a study of their relation to the canker problem shows that the fungous flora of the Citrus canker perhaps may be an interesting problem in itself.

The organism appears to be a new species and is briefly described as follows:

Pseudomonas citri, n. sp.

This organism is a short, motile rod with rounded ends and a polar flagellum. It occurs singly or in pairs and varies in shape from a short, ellipsoidal form to the typical rod. Its dimensions show corresponding differences, but rod forms usually are 1.5 to 2 by 0.5 to 0.75μ.

When plated out on beef agar at room temperature, the organism appears at the end of 36 to 48 hours, the colonies showing up as fine, glistening points just visible to the naked eye. The surface colonies increase quite rapidly in size and in three or four days show very distinctly. They are circular in outline, with entire margins and a slightly raised, smooth surface. By reflected light the colonies show a dull yellowish color, while a bluish translucent color is observed by transmitted light. The internal structure is finely granular and the motility of the organism can sometimes be noted in the outer border of the colony by examining the culture under the low power of the microscope.

In needle-stroke cultures on beef agar a moderate filiform growth is produced which does not penetrate the agar. The streak widens slowly and spreads more at the base of the slant surface. The bacterial mass is slightly raised, smooth, shining, and dull yellow in color.

A very characteristic growth is obtained on potato cylinders. In young cultures the organism follows the line of the streak and produces a somewhat raised, shining growth which has a bright-yellow color. A narrow, white zone is noted on the uninfected surface of the potato, following the margin of the bacterial mass. This feature does not persist very long, as the organism grows vigorously on this medium and soon the entire surface of the cylinder is covered with a thick, yellow, shining, viscid mass.

Beef bouillon shows a visible growth in 24 hours. In older cultures a yellow ring is formed at the surface.

Litmus milk shows a deeper blue color, the casein is precipitated, and the clear supernatant liquid appears a deep reddish color when viewed by transmitted light.

Gelatin is liquefied, the line of puncture is filiform, and the growth of the organism takes place at the surface of the culture.

Dunham's solution shows more or less clouding, the heaviest growth taking place in the open end of the tube, where a flocculent growth is noted at the surface. No traces of indol were noted.

This organism produces no gas in the presence of Dunham's solution in combination with dextrose, lactose, or mannit. The organism grows well in all these combinations, especially at the open end of the tube, where a flocculent growth is produced. Dextrose appears to favor the development of this organism particularly, as a heavy, flocculent growth is formed throughout the entire tube. It grows but sparingly in Ushinsky's solution, and in starch-nitrate solution does not reduce the nitrate. The organism grows best under aerobic conditions.

The organism stains readily with carbol fuchsin, and flagella have been demonstrated by means of the methods of Van Ermengem and Dr. Hugh Williams. (See Pl. IX, fig. 2.)

Much confusion and uncertainty seem to exist in the minds of Citrus growers and others in regard to the identification of the true Citrus canker. Many specimens supposed to be infected with canker which have been sent for identification have been found to be injured by fungi or some other cause. A most careful and detailed comparative study of Citrus canker and other diseases resembling it must be made in order to clear up the canker problem and reduce the necessity of frequent bacteriological diagnoses.

Although this paper gives only a very brief account of the etiology of the Citrus canker and many important facts in the life history of the causal organism remain to be determined, the immediate publication of this preliminary report is considered necessary on account of the great economic significance of this disease, which up to the present has been supposed to be due to a fungous parasite. Because the methods of control for bacterial diseases differ quite radically from those employed for fungous diseases it is hoped that the presentation of this report at this early stage in the investigation will lead to a more adequate understanding of the precautions which may be essential in an effective campaign of eradication.

PLATE IX

Pseudomonas citri

Fig. 1.—Drawing of a stained section of a portion of a grapefruit leaf bearing a young canker resulting from inoculation with a pure culture of *P. citri*. × 250.

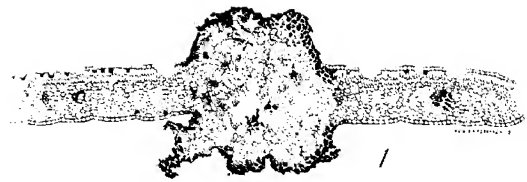
Fig. 2.—Photomicrograph of *P. citri* stained by the Williams method for flagella. × 1,000.

Fig. 3.—Top view of a grapefruit seedling showing the results of artificial inoculation with *P. citri* isolated from Texas specimens.

Fig. 4.—View of the lower side of the leaves shown in figure 3.

Fig. 5.—Top view of a grapefruit seedling showing the results of inoculation with *P. citri* obtained from Florida specimens.

Fig. 6.—View of the lower side of the leaves shown in figure 5.



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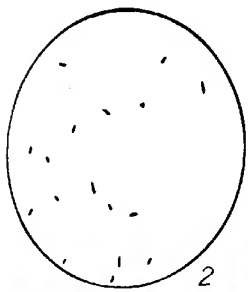

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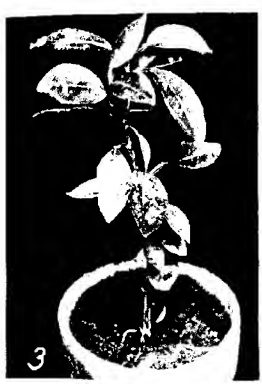

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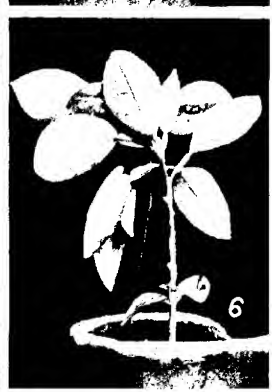

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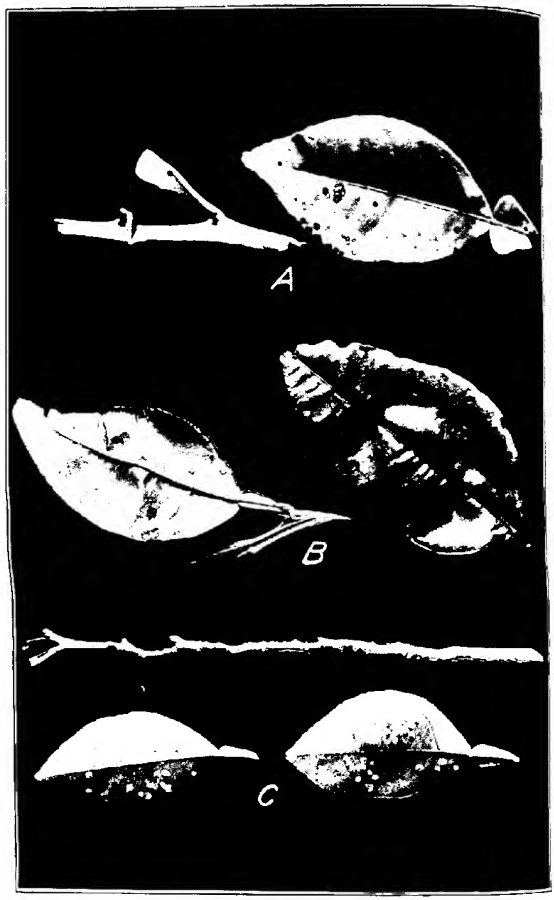
A
B
C

PLATE X

Pseudomonas citri: Small lesions on Citrus twigs and more obvious cankers on Citrus leaves. *A*, Cankers on twig and leaves from Florida produced by natural infection; *B*, natural infections on leaves from Texas; *C*, cankers on twig and leaves produced by artificial inoculation.

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